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Genomische, molekulargenetische und phänotypische Analysen zu Robustheit und Resistenz am Beispiel endoparasitärer Infektionen bei Milchkühen

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ABKÜRZUNGSVERZEICHNIS

ALCAM	Activated leukocyte cell adhesion molecule
AMIR	Antibody mediated immune response
BCS	Body condition score
BHB	ß-Hydroxybutyrate
BTA	Chromosom vom Rind (*Bos taurus*)
CAMs	Cell adhesion molecules
CM	Clinical mastitis
CMIR	Cellular mediated immune response
DD	Dermatitis digitalis
DIM	Days in milk
DS1	Dataset 1
DS2	Dataset 2
DS3	Dataset 3
DSN	Deutsches Schwarzbuntes Niederungsrind (Black and White dual-purpose cattle)
EBV	Estimated breeding value
EC	Electrical conductivity
ELISA	Enzyme-linked immunosorbent assay
EpG	Eizahl pro Gramm Kot
FEC	Faecal egg count
FEC-GIN	Faecal egg count for gastrointestinal nematodes
FEC-FH	Faecal egg count for *Fasciola hepatica*
FEQ	Fett-Eiweiß-Quotient
FH-INF	Infection with *Fasciola hepatica* (binary trait; infected/non-infected)
FLC-DV	Faecal larvae count for *Dictyocaulus viviparus*
FLC	Faecal larvae count
FPR	Fat-to-protein ratio
FTIR	Fourier Transform Infrarot (Spektrometrie)
GI	Generalized Immunity
GIN	gastrointestinale Nematoden
GIN-INF	Infection with gastrointestinal nematodes (binary trait; infected/non-infected)
GPC	Glycerophosphocholine

GUI	Genotyp-Umwelt-Interaktion
GWAS	Genome-wide association study
G x E	Genotype-by-environment (interaction)
HF	Holstein-Friesian
HWE	Hardy-Weinberg-Equilibrium
Ig	Immunoglobulin
IGF-1	Insulin growth factor 1
IL	Interleukin
IR	Immune response
LD	Linkage disequilibrium
MAF	Minor allele frequency
MDS	Magen-Darm-Strongyliden
MFA	Milk fatty acids
MIR	Mid-infrared (technology)
MUN	Milk urea nitrogen
MY	Milk yield
NEFA	Non-esterified free fatty acids
OAR	Chromosom vom Schaf (*Ovies aries*)
PERS	Persistency
QTL	Quantitative trait loci
rFEC-GIN	Residuals of the trait ‘Faecal egg counts for gastrointestinal nematodes’
rFEC-FH	Residuals of the trait ‘Faecal egg count for *Fasciola hepatica*’
RFI	Residual feed intake
rFH-INF	Residuals of the trait ‘Infection with *Fasciola hepatica* (FH-INF)’
rFLC-DV	Residuals of the trait ‚Infection with *Dictyocaulus viviparus*’
rGIN-INF	Residuals of the trait ‘Infection with gastrointestinale nematodes (GIN-INF)’
rMY	Residuals of the trait ‘Milk yield’
ROI	Regions of interest
rSCS	Residuals of the trait ‘Somatic cell score’
SCS	Somatic cell score (dt.: transformierte somatische Zellzahl)
SNP	Single nucleotide polymorphism
spp.	lat.: *species*
THI	Temperature humidity index
ZW	Zuchtwert

ZUSAMMENFASSUNG

In den letzten Jahrzehnten fokussierte die Milchrinderzucht vordergründig auf eine Verbesserung in den Leistungsmerkmalen, wohingegen einer Selektion auf funktionale Merkmale und Robustheit weniger Beachtung geschenkt wurde. Eine gute Robustheit und verbesserte Resistenz gegen wirtschaftlich bedeutende Erkrankungen erlangen seit einigen Jahren jedoch einen zunehmenden Stellenwert in der Milchrinderzucht. Robustheit reflektiert die gute Anpassung der Milchkuh an eine weite Bandbreite von Umweltfaktoren bei einer gleichzeitig guten Widerstandsfähigkeit gegen pathogene Erreger oder metabolische Imbalancen. Gesundheitsmerkmale auf Basis von Dokumentationen der Landwirte zu Euter- oder Klauenerkrankungen werden inzwischen in vielen Ländern in den Selektionsindex integriert. Zudem werden Hilfsmerkmale wie die somatische Zellzahl als Indikator für Eutererkrankungen genutzt. Zunehmend beschäftigt sich die Forschung mit den zugrunde liegenden genetischen Mechanismen für neue innovative Merkmale wie z.B. den Milchketonkörpern ß-Hydroxybutyrat (BHB) und Aceton oder Milchfettsäuren als Indikatoren für Stoffwechselerkrankungen, um diese zukünftig in der Zuchtwertschätzung zu berücksichtigen. Genetische Studien zu infektiösen Erkrankungen, welche durch bakterielle, virale, fungale oder parasitäre Erreger induziert sind, liegen bis dato nicht ausreichend vor. Die notwendige Erfassung aussagekräftiger Phänotypen, der starke Einfluss durch äußere Umwelteinflüsse (z.B. Klima) sowie individuelle Wirt-Pathogen-Interaktionen erschweren genetische Analysen auf diesem Gebiet. Dennoch ist die züchterische Bearbeitung einer genetischen Resistenz gegen infektiöse Erkrankungen beim Milchrind von zentraler Bedeutung, da Zunahmen in der Erregerresistenz gegen derzeit verfügbare Wirkstoffe (z.B. Antibiotika, Anthelminthika) global zu beobachten sind und die Therapiemöglichkeiten damit erschweren.

Der erste Teil der vorliegenden Arbeit beschäftigt sich mit Phänotypisierungsstrategien und mit der Schätzung quantitativ-genetischer Parameter zu konventionell in der Zucht genutzten und innovativen neuen Merkmalen beim Milchrind. Der Schwerpunkt liegt dabei auf Merkmalen, die mit Robustheit und insbesondere mit einer verbesserten Krankheitsresistenz gegen bakterielle und parasitäre Erreger bei der Milchkuh assoziiert sind. Am Beispiel endoparasitärer Infektionen als innovative Merkmale der Krankheitsresistenz und Robustheit beim Milchrind wurden im weiteren Verlauf der vorliegenden Arbeit Studien auf genomischer, molekulargenetischer und phänotypischer Ebene durchgeführt. Die Arbeit gliedert sich in 6

Kapitel, wobei die Kapitel 3, 4 und 5 statistische Analysen zu Endoparasiteninfektionen beim Milchrind unter Einbezug verschiedener Datensätze und verschiedener Selektionslinien schwarzbunter Milchkühe (Deutsches Schwarzbuntes Niederungsrind (DSN), Holstein Friesian (HF), HF-Kreuzungen) beinhalten.

Kapitel 1 gibt einleitend einen Überblick über die Verwendung konventionell genutzter und neuer Merkmale in der Milchrinderzucht, um Robustheit abzuleiten. Weiterhin werden die Begrifflichkeiten Robustheit, Resistenz, Toleranz und Widerstandsfähigkeit terminologiert. Darüber hinaus gibt Kapitel 1 eine Übersicht zu den wirtschaftlich bedeutendsten Endoparasiteninfektionen beim Milchrind sowie über die in der Zucht genutzten Merkmale, um eine Endoparasitenresistenz im Wirt zu messen. Zudem wird einleitend eine Übersicht über bisherige genetische Studien zur Endoparasitenresistenz beim Rind und über die Bedeutung der Zucht auf Endoparasitenresistenz speziell für vom Aussterben bedrohte Rassen mit kleiner Populationsgröße wie das DSN gegeben, da die genomischen und molekulargenetischen Berechnungen in der vorliegenden Arbeit auf das DSN fokussieren. Das DSN ist eine der Gründerrassen der heutigen HF Population und gilt als besonders robust und gut angepasst an Weideproduktionsysteme.

Kapitel 2 addressiert Phänotypisierungsstrategien für konventionelle und neue Merkmale bei Milchkühen, welche in der Zucht als Indikatoren zur Messung der Robustheit genutzt werden. Weiterhin beschäftigt sich Kapitel 2 mit der Schätzung quantitativ-genetischer Parameter für Merkmale, die mit den Begrifflichkeiten Robustheit, Widerstandsfähigkeit, Resistenz und Toleranz assoziiert sind und welche im Rahmen züchterischer Selektionsstrategien genutzt werden und zukünftig genutzt werden können. Der Fokus liegt dabei insbesondere auf neuen, innovativen Merkmalen wie bakteriellen und endoparasitären Infektionsmerkmalen auf Basis labordiagnostischer Tests. Es wird einleitend ein Überblick über züchterische Strategien wie die Ermittlung von Genotyp-Umwelt-Interaktionen (GUI) geliefert, welche klassischerweise genutzt werden, um Unterschiede in den Genotypen für gleiche Merkmale in verschiedenen Haltungsumwelten abzuleiten und somit „robustere Genotypen“ zu identifizieren. Zudem wird über die von Landwirten erfassten Diagnose- bzw. Gesundheitsdaten und deren Inkludierung in den Selektionsindex diskutiert. Neben den Diagnosedaten finden zudem weitere Indikatoren, sogennante Biomarker, Anwendung in der Zucht. Die in Kapitel 2 vorgestellten Indikatoren beinhalten Biomarker zur Identifizierung von Eutererkrankungen und Stoffwechselerkrankungen sowie Indikatoren für Hitzestress, die ruminale Mikrobiomzusammensetzung und endoparasitäre Infektionen, welche im Sinne einer Zucht zur Verbesserung der Krankheitsresistenz beim Rind genutzt werden können. Schätzungen

quantitativ-genetischer Parameter für endoparasitäre Infektionen ergaben niedrige Heritabilitäten für Infektionsmerkmale von Nematoden (Rundwürmern), jedoch Heritabilitäten im moderaten Bereich für Infektionsmerkmale von Trematoden (Saugwürmer). Dieses Ergebnis zeigt, dass die Wirtsresistenz und Wirt-Parasiten-Interaktionen erregerspezifisch sind. Im letzten Abschnitt von Kapitel 2 wird die praktische Anwendung von Immunmerkmalen in der Milchrinderzucht erläutert und deren Beziehung mit Leistungsmerkmalen und funktionalen Merkmalen diskutiert. Insbesondere für die verbesserte Resistenz gegen bakterielle, virale oder parasitäre Erreger beim Rind ist die Selektion auf eine verbesserte zelluläre sowie humorale Immunantwort von züchterischem Interesse.

Kapitel 3 baut auf den Schätzungen quantitativ-genetischer Parameter für endoparasitäre Infektionen aus Kapitel 2 auf. Bei Milchkühen spielen neben den gastrointestinalen Nematoden (GIN) und dem Rinderlungenwurm *Dictyocaulus viviparus* der große Leberegel *Fasciola hepatica* eine wirtschaftliche Bedeutung. In Kapitel 3 wurde aufbauend auf den Erkenntnissen der quantitativ-genetischen Studien zu Endoparasitenmerkmalen eine genomweite Assoziationstudie (GWAS) zur Identifizierung von SNP-Markern und Kandidatengenen, welche mit einer Endoparasitenresistenz in Verbindung stehen, durchgeführt. Ein weiteres Ziel war die Schätzung von SNP-Effekt Korrelationen zwischen Endoparasitenmerkmalen und den Merkmalen Milchleistung und transformierte somatische Zellzahl (engl.: Somatic cell score, SCS) innerhalb identifizierter interessanter genomischer Regionen. Damit knüpft Kapitel 3 direkt an die vorangegangenen Studien auf quantitativ-genetischer Ebene an. Die GWAS wurde in einer Subpopulation von 148 Kühen der Rasse DSN durchgeführt, welche bereits in die quantitativ-genetischen Analysen inkludiert waren. Beim DSN handelt es sich um eine vom Aussterben bedrohte Rasse mit kleiner Populationsgröße. Da DSN eine besonders gute Angepasstheit und Robustheit an die Bedingungen auf der Weide nachgesagt wird, ist die Analyse bedeutender Merkmale wie z.B. eine verbesserte Endoparasitenresistenz für den Erhalt der Population von züchterischer Relevanz. Für DSN lag in der vorliegenden Studie nur eine begrenze Anzahl an Endoparasitenphänotypen vor. Daher wurde ein zweistufiger Analyseansatz für die GWAS gewählt. In einem ersten Schritt wurde mittels linear gemischter Modellanalysen in dem Ursprungsdatensatz von 1.166 schwarzbunten Milchkühen der Rassen HF, HF x neuseeländische HF, HF-Kreuzungen und DSN auf fixe Effekte für Merkmale patenter Endoparasiteninfektionen vorkorrigiert. Bei den Merkmalen handelte es sich um die Eiausscheidung für GIN und *F. hepatica*, sowie um die Larvenausscheidung des Rinderlungenwurmes, *D. viviparus*, mit dem Kot. Im zweiten Schritt der Analysen wurden die vorkorrigierten Endoparasitenphänotypen (Residuen) als abhängige Merkmale unter Einbezug

von 423,654 SNPs der 148 DSN Kühe in der GWAS analysiert. Insgesamt wurden 44 SNPs über der genomweiten Signifikanzschwelle (p_{Bonf} = 4.47 x 10^{-7}) identifiziert, wohingegen 145 Marker über den chromosomenweiten Signifikanzschwellen (7.47 x 10^{-6} auf BTA 1 bis 2.18 x 10^{-5} auf BTA 28) lagen. Insgesamt wurden 23 Kandidatengene mit den assoziierten SNPs in Verbindung gebracht. Für *F. hepatica*-Infektionen wurde das Activated leukocyte cell adhesion molecule (*ALCAM*) Gen auf BTA 1 als Kandidatengen identifiziert. Davon wurde nur ein Gen (*NAV3*; Neuron navigator 3) für zwei verschiedene Endoparasitenmerkmale (GIN- und *D. viviparus* Ei/Larvenausscheidung) identifiziert. Dies deutet darauf hin, dass die in eine Resistenz involvierten Gene spezifisch für den jeweiligen Erreger sind. Weiterführend konnten vier „Pathways" identifiziert werden, welche in Immunfunktionen bzw. Wirt-Parasiten-Interaktionen, involviert sind. Die SNP-Effekt Korrelationen innerhalb der einbezogenen genomischen Regionen zeigen, dass eine Zucht auf GIN- und *F. hepatica* Resistenz nicht mit einer erhöhten Anfälligkeit für bakterielle Euterinfektionen (Indikator: somatische Zellzahl) assoziiert ist.

In **Kapitel 4** wurden weiterführend Sequenzanalysen des *ALCAM* Gens für Untersuchungen zur Endoparasitenresistenz beim DSN durchgeführt. Im Rahmen der in Kapitel 3 durchgeführten GWAS wurde der Chip-SNP *ALCAMc.73+32791A>G* in dem Kandidatengen *ALCAM* für das Zelladhäsionsmolekül ALCAM (engl.: activated leukocyte cell adhesion molecule) mit einer Resistenz für *F. hepatica* beim DSN assoziiert. Das Gen *ALCAM* codiert für das Zelladhäsionsmolekül CD166/ALCAM, welches zu den Immunoglobulinen zählt und als Ligand für das Molekül CD6 fungiert, einen Signalrezeptor für T- und B-Zellen. Die Interaktion zwischen CD166/ALCAM und CD6 nimmt eine Schlüsselrolle in der Immunabwehr gegen eine Vielzahl von Erkankungen ein, was u.a. in Studien beim Menschen und Rind gezeigt wurde. Daher fokussierten die molekulargenetischen Analysen dieses Kapitels auf das Gen *ALCAM* als besonders interessantes Kandidatengen. Die Hypothese ist, dass bestimmte SNPs im *ALCAM* Gen aufgrund der besonderen Bedeutung in der Immunabwehr möglicherweise auch einen Einfluss auf weitere Leistungsmerkmale oder auch auf Merkmale einer Krankheitsresistenz haben. Bei den ausgewählten Merkmalen als Indikatoren für Robustheit und Krankheitsresistenz handelte es sich um die bereits in Kapitel 2 und/oder Kapitel 3 addressierten Indikatoren wie Fett-Eiweiß-Quotient (FEQ), SCS und Laktationspersistenz, welche mit dem monatlichen Testtag der Kühe erfasst werden oder abgeleitet werden können. Zudem wurde in diesem Kapitel auf das mit einer *F. hepatica*-Resistenz assoziierte *ALCAM* Gen fokussiert, da bedeutende ökonomische Verluste durch *F. hepatica*-Infektionen verzeichnet werden und die Therapiemöglichkeiten beim Milchrind

stark limitiert sind. Analysen auf genomischer und molekulargenetischer Ebene sind daher von von großer Relevanz, um genetische und immunologische Mechanismen einer *F. hepatica*-Resistenz beim Rind zu verstehen und in zukünftigen Selektionsstrategien oder Entwicklungen spezieller SNP-Chips berücksichtigen zu können. Ziel war eine Sequenzierung des in der GWAS identifizierten *ALCAM* Gens sowie die Schätzung von Allelsubstitutionseffekten und Dominanzeffekten für Merkmale einer Endoparasitenresistenz, Leistungsmerkmale sowie weitere Testtagsmerkmale, die mit einer Krankheitsresistenz assoziiert sein können.

Die Sequenzierung des *ALCAM* Gens erfolgte in einem Case-Control-Design unter Einbezug von 5 DSN mit den höchsten *F. hepatica*-Eiausscheidungsraten und 5 DSN ohne *F. hepatica*-Eiauscheidung, erfasst mittels wiederholter koproskopischer Untersuchungen. Im Rahmen dieser Sequenzanalysen wurde ein signifikanter Unterschied in der Allelfrequenz für einen SNP in *ALCAM* Exon 9 identifiziert. Daraufhin wurden weitere 84 DSN für Exon 9 und die flankierenden intronischen Bereiche sequenziert. Die in diesem Datensatz von insgesamt 94 DSN identifizierten kausalen Mutationen befanden sich in Exon 9 (*ALCAMc.1017T>C*) sowie in Intron 9 (*ALCAMc.1104+10T>A, ALCAMc.1104+85T>C*). In einem nächsten Schritt sollten die drei Mutationen sowie der zuvor in der GWAS detektierte SNP *ALCAMc.73+32791A>G* in Zusammenhang mit den Merkmalen *F. hepatica*-Infektion (FH-INF), GIN-Infektion (GIN-INF), Milchleistung, Milchfettgehalt, Milchproteingehalt, FEQ, SCS und Laktationspersistenz analysiert werden. Die Assoziationen zwischen den vorkorrigierten Endoparasitenphänotypen (= Residuen; rFH-INF, rGIN-INF) einer *F. hepatica* oder GIN-Infektion sowie den Zuchtwerten (ZW) für alle weiteren Merkmale wie Milchleistung, Milchfett- und Milchproteingehalt, FEQ und SCS wurden mittels logistischer Regressionsanalyse berechnet. Mit der logistischen Regressionsanalyse wurde die Wahrscheinlichkeit für das Auftreten des heterozygoten Genotyps in Abhängigkeit des Zielmerkmals berechnet. Die Allelsubstitutionseffekte für das Merkmal rFH-INF waren für alle vier Loci signifikant. Das T Allel der SNPs *ALCAMc.1017T>C* und *ALCAMc.1104+85T>C* stellte sich als vorteilhaft für eine *F. hepatica*-Resistenz heraus. Für das Merkmal rGIN-INF wurde eine signifikante Allelsubstitution lediglich für den Chip-SNP *ALCAMc.73+32791A>G* detektiert. Signifikante Assoziationen zeigten sich zudem zwischen den identifizierten SNPs und den ZW für die Merkmale Milchfett-und Milchproteingehalt sowie dem FEQ. Die Dominanzeffekte für die ZW der Testtagsmerkmale lagen zwischen 0,00 und 0,47 SD und für die Endoparasitenmerkmale zwischen 0,08 und 0,82 SD. Günstige Dominanzeffekte für gleiche Genotypen zeigten sich zwischen den Merkmalen rFH-INF und dem FEQ, wohingegen gegensätzliche Dominanzeffekte zwischen rFH-INF und SCS berechnet wurden. Mittels einer Kombination

aus Sequenzanalyse des *ALCAM* Gens mit dem selektiven Genotypisierungsansatz ließen sich Polymorphismen in dem Gen in DSN identifizieren, welche nicht nur eine *F. hepatica*-Resistenz, sondern auch weitere Leistungsmerkmale beeinflussen. Die identifizierten und analysierten SNPs könnten zukünftig im Rahmen eines speziellen, für das DSN entwickelten SNP-Chips genutzt werden. Die in dieser Studie gewonnenen Erkenntnisse beim DSN bieten zudem einen wissenschaftlichen Ansatz für eine weitere Validierung in anderen Rinderpopulationen.

In Kapitel 1 bis 4 wurden die wirtschaftliche Relevanz endoparasitärer Infektionen beim Milchvieh und dabei insbesondere die ökonomische Bedeutung von *F. hepatica*-Infektionen herausgestellt. **Kapitel 5** greift die aktuelle Bedeutung von *F. hepatica*-Infektionen in norddeutschen Milchviehherden durch Seroprävalenz-Analyse sowie den Einfluss von *F. hepatica*-Infektionen auf weitere ausgewählte Produktions- und funktionale Merkmale auf. Phänotypische Assoziationsanalysen zwischen *F. hepatica*-Antikörpertitern in der Tankmilch mit den Milchleistungsmerkmalen Milch-kg, Milchfett- sowie Milchproteingehalt auf Herdenebene bilden den Schwerpunkt von Kapitel 5. Desweiteren wurde die Beziehung zwischen *F. hepatica*-Herdenantikörpertitern und der somatischen Zellzahl beleuchtet, um die Ergebnisse der quantitativ-genetischen Vorstudien, der genomischen Studien aus Kapitel 3 und der molekulargenetischen Studien aus Kapitel 4 auf phänotypischer Ebene zu bestätigen oder widerlegen zu können. Als Basis für die Analysen diente ein Datensatz von 1.022 auf *F. hepatica*-Antikörper untersuchte Herden im Herbst 2017 sowie 1.318 Herden im Herbst 2018. Seroprävalenzstudien zu *F. hepatica* wurden letztmalig in 2006, 2008 und 2010 in Deutschland sowie für die Region Ostfriesland veröffentlicht. Die ermittelte Seroprävalenz betrug 33,1 % im Herbst 2017 und 37,0 % im Herbst 2018 und lag damit niedriger als die Seroprävalenz in Vorstudien aus den Jahren 2010, 2008 und 2006 (> 45 % positive Herden). Die Assoziationsanalysen ergaben eine signifikant negative Beziehung zwischen der *F. hepatica*-Herdenantikörpertiter-Kategorie und der durchschnittlichen täglichen Milchleistung mit 1,62 kg Milchverlust pro Kuh pro Tag in den stark infizierten Herden im Vergleich zu den ELISA-negativen Herden. Auch zeigte die Infektionskategorie der Herden einen signifikanten Effekt auf den Milchprotein- und Milchfettgehalt mit einem Verlust von 0,06 kg für beide Parameter in stark infizierten Herden im Vergleich zu ELISA-negativen Herden. Zwischen *F. hepatica*-Herdenantikörpertiter-Kategorie und der somatischen Zellzahl ergab sich kein signifikanter Zusammenhang in den Analysen.

Erstmalig wurde zudem die Assoziation zwischen *F. hepatica*-Herdenantikörpertitern und den aus der Milch mittels Fourier Transform Infrarot (FTIR)-Spektrometrie abgeleiteten

Ketonkörpern Betahydroxybutyrat (BHB) und Aceton, welche bereits in Kapitel 2 als Biomarker zur Detektierung einer primären Ketose behandelt wurden, geschätzt. Die Hypothese ist, dass *F. hepatica*-Infektionen durch die im Wirt hervorgerufene Leberschädigung die Bildung von Glucose reduzieren und damit sekundär zu einer erhöhten Bildung von Ketonkörpern im Tier führen. Dieser physiologische Zusammenhang wurde bereits in Studien beim kleinen Wiederkäuer nachgewiesen. Die durchschnittlichen Herdenwerte für BHB und Aceton wurden auf Basis wiederholter Beobachtungen für BHB und Aceton von Einzeltieren zwischen Laktationstag 5 und 60 gebildet. In der vorliegenden Arbeit konnte ein signifikanter Zusammenhang zwischen *F. hepatica*-Herdenantikörpertiter-Kategorie und den durchschnittlichen BHB-Werten in der Milch geschätzt werden. Herden mit der höchsten Infektionskategorie zeigten signifikant höhere durchschnittliche BHB-Werte im Vergleich zu Herden in den anderen Infektionskategorien. Für die durchschnittlichen Aceton-Werte in der Milch zeigte sich keine signifikante Beziehung zu der *F. hepatica*-Herdenantikörpertiter-Kategorie. Kapitel 5 zeigt damit beispielhaft wie wichtig es ist, die in der Zucht genutzten Indikatormerkmale wie beispielsweise die Erhöhung von Milchketonkörpern zur Diagnose primärer Ketosen, in einem tierärztlichen Gesamtkontext zu betrachten und mögliche Differentialdiagnosen als Ursachen für phänotypische Abweichungen einzelner Indikatormerkmale in Betracht zu ziehen.

Kapitel 6 fasst in einem allgemeinen Diskussionsteil die wichtigsten Ergebnisse und Erkenntnisse der wissenschaftlichen Studien aus Kapitel 3 bis 5 zusammen. Zudem wird ein Ausblick zu züchterischen Selektionsstrategien zur Verbesserung der Endoparasitenresistenz unter Einbezug aktueller sowie neuer genetisch-statistischer und molekulargenetischer Methoden gegeben.

SUMMARY

Over the last few decades, dairy cattle breeding has focussed on improving performance, while paying less attention to functional traits and robustness. However, improved robustness and resistance to economically significant diseases gained increasing importance in dairy cattle breeding for several years. Robustness reflects best adaptation to a broad range of environments by a simultaneous high resilience against pathogenic agents or metabolic imbalances. In many countries, producer-recorded health data (e.g., mastitis diagnosis, claw disorders) are included in the selection index. Furthermore, indicator traits such as the somatic cell score are used as a diagnostic parameter for udder health. Increasingly, research is looking at the underlying genetic mechanisms for novel innovative traits (e.g., milk ketone bodies Betahydroxybutyrate (BHB) and acetone or milk fatty acids) as indicators for metabolic disease, and for the inclusion in genetic evaluation. Genetic studies addressing infectious diseases induced by bacterial, viral, fungal or parasite pathogens are limited. Limitations are given by the lack of large phenotype datasets, the strong impact of environmental factors (e.g., climatic factors) as well as host-pathogen interactions. However, increased resistance of pathogens against actually approved anthelmintics and antibiotics was observed globally, resulting in complications for treatment options. Hence, genetic studies addressing resistance to infectious diseases in dairy cows are of great importance.

The first part of this thesis deals with phenotyping strategies and with the estimation of quantitative-genetic parameters for conventional and innovative novel traits in dairy cattle breeding. The main focus is on traits being associated with robustness and improved disease resistance against bacterial and parasitic agents in dairy cows. Using the example of endoparasite infections as innovative traits associated with disease resistance and robustness in dairy cattle, genomic, molecular-genetic and phenotypic studies were carried out in the course of the work. The thesis is divided into 6 chapters, with chapter 3, 4 and 5 containing statistical analyses for endoparasite infections in dairy cattle by including different datasets with different genetic lines of Black and White dairy cattle (Black and White dual-purpose cattle (DSN), Holstein Frisian (HF), HF crossbreds).

Chapter 1 gives an overview about the development and application of conventional and novel traits in dairy cattle breeding, especially with regard to robustness. In this regard, the terminologies robustness, resistance, tolerance and resilience will be explained. Furthermore, chapter 1 provides an overview for endoparasite infections in dairy cattle and for indicator traits to measure endoparasite resistance in ruminants. Additionally, chapter 1 gives a retrospective

review of previous genetic studies on endoparasite resistance. Since we focussed on the endangered DSN breed in the genomic and molecular-genetic studies, breeding for endoparasite resistance in small endangered breeds will be discussed. The DSN is the founder breed of the modern HF cattle and defined as robust under harsh environmental and pasture conditions.

Chapter 2 addresses phenotyping strategies for conventional and novel traits in dairy cows, which are used in breeding as indicators to measure robustness. Furthermore, chapter 2 deals with the estimation of quantitative-genetic parameters for traits linked to robustness, resistance, tolerance and resilience in dairy cattle breeding. Innovative and novel traits such as bacterial and endoparasitic infection traits from laboratory diagnostics are one main focus of chapter 2. Moreover, chapter 2 gives an overview about classical breeding approaches for improved robustness, including genotype-by-environment (G x E) interactions and for the inclusion of producer-recorded health data into selection indices. Beside producer-recorded data, indicator traits or biomarkers are used in cattle breeding aiming on improved robustness. Indicators addressed in chapter 2 include biomarkers for the identification of udder or metabolic diseases and biomarkers to measure heat stress, the rumen microbiome composition or endoparasite resistance to improve overall disease resistance. Estimations of quantitative-genetic parameters for endoparasite infections resulted in low heritabilities for nematode (roundworm) infection traits, but moderate heritabilities for trematode (flukes) infections traits. Hence, expression of resistance in the host is pathogen-specific. In the last section of chapter 2, the application of immune response traits in dairy cattle breeding and their relationship with milk production traits and functional traits will be discussed. Breeding for improved cellular and humoral immune response mechnisms in cattle is of great importance for improved resistence to infectious pathogens.

Chapter 3 bases on the results from chapter 2, focussing on the estimation of quantitative-genetic parameters for endoparasite infections in dairy cattle. Beside gastrointestinal nematodes (GIN) and the bovine lungworm (*Dictyocaulus viviparus*), infections with the liver fluke *Fasciola hepatica* have high economic impact in dairy cattle. Based on the results from the quantitative-genetic studies for endoparasite resistance traits, we applied a genome-wide association study (GWAS) in chaper 3 in order to identify genetic markers and potential candidate genes being involved in endoparasite resistance. A further objective was to estimate SNP effect correlations between endoparasite traits with the traits milk yield and the logarithmic transformated somatic cell score (SCS). The GWAS was applied in an endangered subpopulation of 148 DSN cattle with a limited number of phenotypic records. DSN are defined as robust cattle under harsh environmental conditions, raising the interest to study

characteristically resistance traits such as endoparasite resistance. We used a 2-step approach for the GWAS. First, endoparasite traits from the population of 1,166 Black and White dairy cows [including Holstein Friesian (HF), HF crossbreds and DSN] were precorrected for fixed effects using linear mixed models. Afterwards, the precorrected phenotypes were the dependent traits in the GWAS based on 423,654 SNPs from 148 DSN cows. We identified 44 SNPs above the genome-wide significance threshold (p_{Bonf} = 4.47 x 10^{-7}), and 145 associations surpassed the chromosome-wide significance threshold (range: 7.47 x 10^{-6} on BTA 1 to 2.18 x 10^{-5} on BTA 28). The associated SNPs identified were annotated to 23 candidate genes. The activated cell adhesion molecule (*ALCAM*) gene on BTA 1 was associated with *F. hepatica* infections. One gene, the Neuron navigator 3 (*NAV3*) gene was associated with resistance to both GIN and *D. viviparus* infections. The DAVID analysis inferred four pathways as being related to immune response mechanisms or involved in host-parasite interactions. SNP effect correlations considering specific chromosome segments indicate that breeding for resistance to GIN or *F. hepatica* as measured by fecal egg counts is genetically associated with a higher risk for udder infections.

Chapter 4 addresses sequence analyses for endoparasite resistance in DSN. The chip SNP *ALCAMc.73+32791A>G* in the activated leukocyte cell adhesion molecule (*ALCAM*) gene was inferred as a candidate for *F. hepatica* resistance in DSN in the GWAS in chapter 3. The *ALCAM* gene encodes for the cell adhesion molecule CD166/ALCAM, an immunoglobulin which acts as a ligand for CD6, a signalling receptor molecule on T and B cells. Studies in humans and cattle reported the key role of CD166/ALCAM and CD6 interaction in immune response to disease. Hence, SNPs in the *ALCAM* gene might influence further resistance and performance traits. Resistance and performance traits of interest included fat-to-protein ratio, SCS and lactation persistency, which were previously addressed in chapter 2 and 3. The aim of chapter 3 was a sequence analysis of the *ALCAM* gene, and to estimate allele substitution and dominance effects for endoparasite resistance, production traits and test-day traits being associated with overall robustness. For *ALCAM* sequencing, a case-control study was designed including 5 DSN with the highest faecal egg counts and 5 DSN with no egg excretion for *F. hepatica*, assessed via coproscopical examinations. In this regard, we identified a significant difference in allele frequency for a SNP in exon 9 of *ALCAM*. Hence, we conducted a sequence analysis for further 84 DSN for exon 9 and the flanking intronic regions. In this regard, causal mutations were identified in exon 9 (*ALCAMc.1017T>C*) and in intron 9 (*ALCAMc.1104+10T>A, ALCAMc.1104+85T>C*) in the subset of 94 DSN cows. In a further step, we analysed the relationship between identified polymorphisms with *F. hepatica*

infections (FH-INF), GIN infections (GIN-INF) and with the estimated breeding values (EBVs) for production and test-day traits. We applied logistic regression analyses for the association between SNP genotypes with residuals for endoparasite traits (rINF-FH, rGIN-INF) and EBVs for production and test-day traits in order to estimate the probability of the heterozygous genotype in dependency of the target trait. Allele substitution effects for rFH-INF were significant for all four loci. The T allele of the SNPs *ALCAMc.1017T>C* and *ALCAMc.1104+85T>C* was the favourable allele when improving resistance against FH-INF. Significant allele substitution for rGIN-INF was only found for the chip SNP *ALCAMc.73+32791A>G*. We identified significant associations between the SNPs with EBVs for milk fat%, protein% and FPR. Dominance effects for the EBVs of test-day traits ranged from 0.00 to 0.47 SD, and were in the direction of improved resistance for rFH-INF. We estimated favourable dominance effects from same genotypes for rFH-INF and FPR, but dominance effects were antagonistic between rFH-INF and SCS. The selective genotyping approach combined with sequence analyses of the *ALCAM* gene revealed SNPs with impact on endoparasite resistance and production traits in DSN. The identified SNPs have to be considered in future selection strategies aming on the development of SNP chips specifically designed for DSN.

The economic impact of *F. hepatica* infections in dairy herds was well discussed in the chapters 1 to 4. Infections with the liver fluke *Fasciola hepatica* remain a serious problem in dairy herds causing significant production losses. Hence, **chapter 5** focuses on *F. hepatica* seroprevalences and on the impact of *F. hepatica* infections measured via herd antibody levels on the production traits milk yield, milk protein and milk fat content in Northern German dairy herds. Moreover, the relationship between *F. hepatica* herd antibody levels and somatic cells in milk was addressed, in order to confirm or to disconfirm the results from the quantitative-genetic, genomic and molecular-genetic studies in chapter 3 and 4. Bulk tank milk (BTM) samples collected between October and December from 1,022 herds in 2017 and from 1,318 herds in 2018 provide the basis for the analyses in chapter 5. Overall, 33.1% of the herds were tested positive in 2017 and 37.0% in 2018, showing decreased *F. hepatica* seroprevalences compared to prior seroprevalence studies in the same region in 2010, 2008 and 2006 (> 45% positive herds). The results of the association analysis showed a significant negative association between herd *F. hepatica* infection category and average milk yield with a loss of -1.62 kg per cow per day in strongly infected herds compared to BTM ELISA negative herds. Moreover, *F. hepatica* infection category had a significant effect on herd average milk protein and fat yield, showing

a decrease of 0.06 kg for both parameters from BTM ELISA negative herds to strongly infected herds. No significant association with somatic cells in milk was found.

For the first time the association between *F. hepatica* herd antibody levels and milk ketone bodies ß-hydroxybutyrate (BHB) and acetone, inferred from Fourier transform infrared (FTIR) spectrometry, was analysed. BHB and acetone were addressed in chapter 2 as biomarkers for primary ketosis. In sheep, a strong relationship between *F. hepatica* infections and an increase in serum ketone bodies due to reduced feed intake and liver damage was demonstrated. Hence, in chapter 5, we hypothesized that *F. hepatica* infections might contribute to an increase in milk ketone bodies in dairy herds. Average herd values for BHB and acetone were computed based on repeated BHB and acetone measurements from individual cows between 5 and 60 days in milk. The results from the association analysis showed significant higher average BHB values in strongly infected herds compared to the other three infection categories in the model analysis. The association between *F. hepatica* infection category and acetone values was not significant. Besides primary ketosis, fasciolosis should be considered as differential diagnosis in dairy herds with increased BHB values.

Finally, **Chapter 6** gives a general discussion focusing on the most pertinent results from chapter 3 to 5. Furthermore, some concerns and outlooks regarding endoparasite resistance traits and breeding strategies for improved endoparasite resistance considering actual and novel genetic-statistical modelling approaches and molecular-genetic technologies are discussed.

KAPITEL 1

Einleitung

Robustheit und die verbesserte Resistenz und Toleranz gegenüber Erkrankungen nehmen in der Milchrinderzucht einen zunehmenden Stellenwert ein (Calus et al., 2013). In den letzten Jahrzehnten wurde klassischerweise auf hohe Milchleistung selektiert, da die Milch trotz schwankender Milchpreise die Haupteinnahmequelle für Milchviehhalter ist. Erst seit einigen Jahren sind funktionale Merkmale und die Zucht auf eine verbesserteTiergesundheit wieder in den Fokus öffentlichen und tierzüchterischen Interesses gerückt (Egger-Danner et al., 2015). Grund dafür ist, dass die Zucht auf hohe Leistung mit physiologischen und immunologischen Imbalancen einhergehen und damit nachteilige Effekte auf die Tiergesundheit haben kann. Physiologische und immunologische Imbalancen treten insbesondere zu Beginn der Laktation in Erscheinung und können eine erhöhte Infektanfälligkeit sowie eine höhere Sensibilität gegenüber Schwankungen in den äußeren Umwelteinflüssen nach sich ziehen (Rauw und Gomez-Raya, 2015). Neben diesem Aspekt tragen die von Verbrauchern und Politik geforderte Verbesserung des Tierwohls, ethische Aspekte, aber auch angestrebte ökonomische Faktoren wie sinkende Kosten in tierärztlichen Behandlungen und Medikamenten dazu bei, dass funktionale Merkmale wieder verstärkt in der Milchrinderzucht Berücksichtigung finden. Im Zuge dieser Debatten um „Tierwohl und Tiergesundheit“ zeichnet sich in Deutschland auch ein zunehmender Trend zur Milcherzeugung in Weideproduktionssystemen ab. Im Gegensatz zu Stallhaltungssystemen verlangen Weideproduktionssysteme den Milchkühen angepasste Eigenschaften wie Robustheit und eine erhöhte Toleranz gegenüber Schwankungen in den Produktions- und Umweltbedingungen auf der Weide ab, was die zunehmende Notwendigkeit züchterischer Strategien für eine verbesserte Robustheit impliziert.

1.1 Zucht auf Robustheit beim Milchrind

Der Begriff ‚Robustheit‘ hat sich während der letzten Jahre in der Milchrinderzucht etabliert und impliziert die gute allgemeine Widerstandsfähigkeit, Resistenz und Toleranz gegen nicht-infektöse sowie infektiöse Erkrankungen sowie die gute Toleranz gegenüber äußeren Einwirkungen wie Schwankungen in den Umwelt- oder Haltungsbedingungen. Zucht auf Robustheit beinhaltet die Identifikation von Tieren, welche unter verschiedensten Umweltbedingungen ein konstantes und hohes Produktionsniveau bei gleichzeitiger Aufrechterhaltung einer guten Gesundheit zeigen (Rauw und Gomez-Raya, 2015).

Züchterische Ansätze zur Identifizierung „robuster Genotypen“ basieren klassischerweise auf Untersuchungen zu Genoyp-Umwelt-Interaktionen (GUI), also Unterschiede in den Genotypen für gleiche Merkmale in verschiedenen Haltungsumwelten. Ein Richtwert ≤ 0,8 für die genetische Korrelation gleicher Merkmale in verschiedenen Umwelten gilt als Indikator für GUI (Robertson, 1959). Methodisch erfolgt die Berechnung genetischer Korrelationen zwischen gleichen Merkmalen in verschiedenen Umwelten mittels Mehr-Merkmals-Modellen, Vatermodellen oder sogenannter ‚Random-Regressionsmodelle‘ bzw. ‚Reaktionsnormmodelle‘ (Calus und Veerkmap, 2003; Haile-Mariam et al., 2008). Reaktionsnormmodelle modellieren den Phänotyp als Polynomialfunktion in Abhängigkeit eines Umweltdeskriptors, um den Einfluss des Genotyps auf die Funktionskoeffizienten (z.B. Steigung) zu untersuchen, wobei eine steile Steigung für eine hohe Umweltsensibilität spricht (Streit et al., 2012). Neben klassischen Umweltdeskriptoren zum Herdenmanagement (z.B. Herdengröße) wurden Studien zu GUI durchgeführt, welche direkt mit einer Krankheitsresistenz assoziierte Merkmale wie Body-Condition Score (BCS), Laktationspersistenz oder Herdenantikörpertiter für parasitäre Infektionen als Umweltdeskriptoren nutzten (Calus und Veerkamp, 2003; Fikse et al., 2003; Twomey et al., 2018). Die Autoren dieser Studien schätzten GUI ≤ 0,8 für Milchproduktions- als auch Fruchtbarkeitsmerkmale und konnten zeigen, dass die Leistungsfähigkeit der Kühe maßgeblich von der Herdengesundheit und einer Exposition mit pathogenen Erregern abhängt.

Züchterische Fortschritte in der Zucht auf eine verbesserte Robustheit und Krankheitsresistenz wurden inbesondere durch die Inkludierung von Gesundheitsdaten in den Selektionsindex erzielt, was inzwischen in vielen Ländern gängige Praxis ist (Egger-Danner et al., 2015). Bei den inkludierten Gesundheitsdaten handelt es sich in der Regel um vom Landwirt erfasste Erkrankungen, welche mittels sogenannter ‚Diagnose- oder Tiergesundheitsschlüssel‘ detailliert dokumentiert werden. Neuenschwander et al. (2012) und Zwald et al. (2004) schätzten niedrige Heritabilitäten zwischen 0,02 und 0,21 für die von Landwirten erfassten Diagnosen zur Tiergesundheit. Die subjektive Erfassung durch den Landwirt sowie niedrige Inzidenzen vieler Erkrankungen erschweren jedoch eine Zuchtwertschätzung auf Basis direkt abgeleiteter Gesundheitsmerkmale (Neuenschwander et al., 2012). Aus diesem Grund rückten sogenannte Biomarker oder Indikatoren als neue funktionale Merkmale zur Erhebung der Tiergesundheit in den letzten Jahren zunehmend in den Fokus genetischer Studien (Egger-Danner et al., 2015). Neue funktionale Merkmale umfassen beispielsweise aus der Milch abgeleitete Parameter oder Substrate wie die somatische Zellzahl, Lactoferrin, Milchfettsäuren, Ketonkörper und Antikörpertiter für diverse Infektionskrankheiten sowie im Blut gemessene

Enzyme, Hormone oder weitere Metabolite. Für viele der aufgezählten Indikatoren liegen die Heritabilitäten im moderaten Bereich und eignen sich damit für eine routinemäßige Nutzung in der Zuchtwertschätzung, um Verbesserungen in Robustheit und Krankheitsresistenz zu erzielen (Pryce et al., 2016; Soyeurt et al., 2012). Neue Technologien wie die Fourier Transform Infrarot (FTIR) Spektrometrie erlauben die Ableitung einer Vielzahl an Phänotypen wie den Milchketonkörpern ß-Hydroxybutyrat (BHB) oder Aceton sowie Milchfettsäuren während der routinemäßigen Milchkontrolle. Milchketonkörper und Milchfettsäuren sind mit einer Vielzahl an Erkrankungen und physiologischen Imbalancen bei der Milchkuh assoziiert (Pryce et al., 2016; Van Haelst et al., 2008). Zudem werden mittels FTIR Spektrometrie abgeleitete Werte für Milchketonkörper inzwischen routinemäßig in Managementtools zur Verbesserung der Herdengesundheit und zur Reduktion von Ketosefällen in der Herde genutzt (z.B. Ketoscreen, CanWest DHI, Guelph, ON, Canada; Ketodetect, CLASEL, France).

Robustheit und Resistenz unterliegen insbesondere den Mechanismen der angeborenen (unspezifischen) Immunantwort als auch der adaptiven (spezifischen) Immunantwort. Daher werden Immunmerkmale und genetische Mechanismen für Variationen in der Immunantwort beim Milchrind bereits seit vielen Jahren erforscht (Detilleux et al., 1994; Thompson-Crispi et al., 2012). Die Selektion auf eine verbesserte Immunantwort spielt insbesondere für infektiöse, durch pathogene Erreger induzierte Erkrankungen eine Rolle, obgleich auch Interaktionen zwischen nicht-infektiösen Erkrankungen und der Immunantwort bei Milchkühen beobachtet wurden (Thompson-Crispi et al., 2013). Eine erhöhte Anfälligkeit für nicht-infektiöse Stoffwechselerkrankungen um den Zeitraum der Geburt wird unter anderem auf die verminderte oder vermehrte Bildung bestimmter Immunzellen zurückgeführt. Eine kanadische Forschergruppe konnte zeigen, dass zelluläre (z.B. T- und B-Zellen) und humorale (Antikörper) Bestandteile des Immunsystems mit Heritabilitäten zwischen 0,19 und 0,54 einer genetischen Komponente unterliegen (Heriazon et al., 2013; Thompson-Crispi et al., 2012). Auf Basis dieser Forschungen wurde die sogenannte High Immune Response (HIR) Technologie entwickelt und auf den Markt gebracht, welche dem Landwirt eine gezielte Auswahl von Bullen mit einer besonders hohen Widerstandsfähigkeit gegen Erkrankungen und damit sekundär eine Verbesserung in anderen Produktionsmerkmalen ermöglichen soll. Die genetischen Korrelationen zwischen den Merkmalen zelluläre und humorale Immunanwort mit Produktions- und Fruchtbarkeitsmerkmalen liegen zwischen -0,23 und 0,21 mit überwiegend günstigen Korrelationen zwischen beiden Merkmalskomplexen (Heriazon et al., 2013; Thompson-Crispi et al., 2012). Wie erste Studien zeigen sind Immunmerkmale insbesondere mit infektiösen Erkrankungen assoziiert, wohingegen die genetischen Korrelationen mit nicht-

infektiösen Erkrankungen deutlich niedriger liegen (Ahn et al., 2006). Inwieweit und ob die Zucht auf eine verbesserte zelluläre und humorale Immunantwort mittels HIR Technologie eine Krankheitsresistenz für eine Vielzahl infektiöser Erreger abdeckt, bleibt noch abzuwarten.

1.2 Resistenz, Toleranz und Widerstandsfähigkeit

Milchkühe sind während der peripartalen Phase besonderen physiologischen Herausforderungen ausgesetzt. Durch die reduzierte Futteraufnahme vor der Geburt und den Anstieg des Energiebedarfs mit dem Beginn der Laktation kommt es zu einer negativen Energiebilanz, die durch die Mobilisierung von Körpermasse kompensiert wird. Fettsäureabbau mit paralleler Bildung von Ketonkörpern ist die Folge, was häufig in subklinischen oder klinischen Ketosen und weiteren Stoffwechselerkrankungen resultiert. Studien beim Rind zeigen, dass die Anfälligkeit für infektiöse Erreger wie Bakterien oder Parasiten insbesondere in der Peripartalperiode durch metabolischen Stress und einer damit verbundenen Schwächung des Immunsystems erhöht ist (Perri et al., 2011; Singh et al., 2008). Nicht-infektiöse und infektiöse Erkrankungen bei Milchkühen können zudem Einbußen in der Milchleistung bedingen, was eine züchterische Bearbeitung und die Inkludierung von Gesundheitsdaten und deren Indikatoren in zukünftige Zuchtziele indiziert. Auch wird die Zucht auf eine verbesserte Widerstandsfähigkeit auf Grund von Änderungen in den klimatischen Bedingungen und eine dadurch bedingte Ausbreitung und Inzidenzzunahme für bestimmte bakterielle, virale, fungale oder parasitäre Erkrankungen immer wichtiger (Gauly et al., 2013).

In der Tierzucht fasst man die drei Begrifflichkeiten Resistenz, Toleranz und Widerstandsfähigkeit unter dem Oberbegriff „Robustheit“ zusammen. Resistenz und Toleranz bezeichnen allgemeinhin die gute Widerstandsfähigkeit gegen äußere schädliche Einflüsse sowie gegen Schwankungen in den Umwelt- und Haltungsbedingungen ohne nachteilige Effekte auf Gesundheit und Leistungsfähigkeit des Organismus zu haben. Resistenz, Toleranz und Widerstandsfähigkeit stehen daher in enger Verbindung zu funktionalen Merkmalen wie Fitness, Persistenz, Melkbarkeit, Temperament, Fruchtbarkeit sowie nicht-infektiösen und infektösen Erkankungen (Calus et al., 2013). In der Infektionsepidemiologie mit Fokus auf parasitäre Erreger folgen die drei Begrifflichkeiten Resistenz, Toleranz und Widerstandsfähigkeit einer klaren, abgegrenzten Definition (Bishop, 2012; Rashidi, 2016):

Resistenz: Die Fähigkeit eines Wirtes, die Zahl an Parasiten, die sich im Organismus etablieren, reproduzieren und überleben, zu vermindern bzw. den Lebenszyklus des Parasiten zu beeinflussen.

Toleranz: Fähigkeit eines Wirtes, trotz Anwesenheit eines Parasiten seine Leistung zu erhalten. Der Wirt zeigt wenige bis keine Erkrankungssymptome trotz einer Infektion. Die Schätzung einer Toleranz impliziert dabei wiederholte Messungen, also eine Messung der Leistung vor einer Infektion sowie nach erfolgter Infektion am gleichen Tier.

Widerstandsfähigkeit: Produktivität eines Wirtes in Anwesenheit einer Infektion. Es wird hierbei nur die Diagnose einer Infektion verwendet, die genaue Wurmbürde oder Maßzahlen einer Resistenz (z.B. Eizahlen im Kot) werden dabei nicht bestimmt.

In Kapitel 2 der vorliegenden Arbeit werden die drei Begrifflichkeiten Resistenz, Toleranz und Widerstandsfähigkeit verwendet, wie sie oben erläutert im klassischen tierzüchterischen Kontext angewendet werden. In den Kapiteln 3 bis 6, wird der Begriff „Resistenz“ im Sinne der infektionsepidemiologischen Definition verwendet. Der Begriff „Toleranz“ wird im Kontext endoparasitärer Infektionen hingegen immer dann verwendet, wenn von den Einflüssen einer endoparasitären Infektion auf Leistungsparameter der Kühe die Rede ist.

1.3 Endoparasiteninfektionen und deren wirtschaftliche Bedeutung in Milchproduktionssystemen

Infektionen mit Endoparasiten (Def.: im Inneren des Wirtes lebende Parasiten) sind von großer wirtschaftlicher Bedeutung und haben einen negativen Einfluss auf die Tiergesundheit. Bei Milchkühen sind Infektionen mit parasitischen Würmern (Helminthen), darunter den Magen-Darm-Strongyliden (MDS), dem Rinderlungenwurm (*Dictyocaulus viviparus*) sowie dem großen Leberegel (*Fasciola hepatica*), in ökologisch sowie konventionell geführten Weidebetrieben von zentraler Bedeutung (Vercruysse et al., 2018). Helminthen rufen bei adulten Rindern in der Regel subklinische Infektionen hervor, dennoch sind negative Auswirkungen auf Milchleistung, Fruchtbarkeit und Produktqualität (z.B. Fleischqualität) beschrieben (Charlier et al., 2007a; Charlier et al., 2009; May et al., 2018).

1.3.1 Infektionen mit gastrointestinalen Nematoden

Bei Rindern sind Angehörige der MDS die häufigsten im Gastrointestinaltrakt parasitierenden Nematoden (Rundwürmer). *Ostertagia ostertagi* (Familie der Trichostrongylidae) ist die häufigste MDS-Spezies beim Rind. In Westeuropa sind bis zu 98 % aller Milchviehherden sowie 89 % aller Einzeltiere infiziert (Bellet et al., 2016; Bloemhoff et al., 2015). Weiterhin treten beim Rind häufig die Spezies *Cooperia* spp. und *Trichostrongylus* spp. auf, jedoch mit niedrigeren Einzeltierprävalenzen bis zu 20 % (Agneessens et al., 2000). Bei MDS-Infektionen

handelt es sich in erster Linie um Weideinfektionen, gekennzeichnet durch eine ausgeprägte Saisondynamik mit dem höchsten Peak der Eiausscheidung im Sommer. Bei adulten Rindern sind in Folge einer erworbenen Immunität deutlich niedrigere Wurmbürden und Eiausscheidungsraten als bei Jungtieren zu erwarten (Nødtvedt et al., 2002). Dennoch können MDS-Infektionen bei adulten, unbehandelten Kühen in einer um bis zu 1,2 kg/Kuh/Tag verringerten Milchleistung sowie in einer verminderten Fruchtbarkeit resultieren und damit zu hohen wirtschaftlichen Verlusten in betroffenen Betrieben führen (Charlier et al., 2007b). Neben MDS-Infektionen kommen beim Rind mitunter auch Infektionen mit anderen gastrointestinalen Nematoden wie dem Zwergfadenwurm (*Strongyloides papillosus*), Haarwürmern (*Capillaria* spp.) oder Peitschenwürmern (*Trichuris* spp.) vor. Daher werden die genannten Nematoden in der vorliegenden Arbeit unter dem Begriff „gastrointestinale Nematoden (GIN)“ subsummiert.

1.3.2 Infektionen mit dem Rinderlungenwurm

Der Rinderlungenwurm *Dictyocaulus viviparus* tritt primär bei Jungrindern als Verursacher der parasitären Bronchitis auf, ist aber auch bei adulten Rindern von nicht zu unterschätzender Relevanz (Ploeger et al., 2002; Schunn et al., 2013). Rinder infizieren sich oral mit den 3. Larven von *D. viviparus* auf der Weide, welche über den Lymph-Blut-Weg im Wirt zur Lunge gelangen. Dort angekommen, entwickeln sich die Larven zu adulten Parasiten und parasitieren in der Lunge des Wirtes. Krankheitsausbrüche als Folge von *D. viviparus*-Infektionen werden immer wieder auch in Milchviehherden berichtet (Holzhauer et al., 2011; Wapenaar et al., 2007). Woolley (1997) schätzten wirtschaftliche Verluste bis zu US $ 40.000, welche infolge eines *D. viviparus*-Ausbruches für einen Betrieb mit durchschnittlich 100 Milchkühen in Folge reduzierter Milchleistung und Tierarztkosten entstehen. Bei adulten Rindern steht das sogenannte Reinfektionssyndrom im Vordergrund, welches durch eine immunvermittelte Entzündungsreaktion mit Hemmung der Entwicklung des Parasiten in der Lunge des Wirtes charaktersiert ist (Holzhauer et al., 2011; Schunn et al., 2012). Bei Rindern verlaufen Reinfektionen daher meist subklinisch (Holzhauer et al., 2011). Dennoch sind Leistungseinbußen in Folge von *D. viviparus*-Infektionen beschrieben. Charlier et al. (2016) und Dank et al. (2015) schätzten einen durchschnittlichen Verlust der Milchleistung zwischen 0,50 und 1,68 kg/Kuh/Tag sowie eine Reduktion im Milchprotein- und fettgehalt in Herden mit hohen *D. viviparus*-Antikörpertitern in der Tankmilch im Vergleich zu seronegativen Herden. May et al. (2018) ermittelten einen signifikanten Verlust von 1,62 kg/Kuh/Tag in der durchschnittlichen Milchleistung in patent mit *D. viviparus* infizierten Milchkühen im

Vergleich zu den nicht infizierten Kontrolltieren. In Irland sowie in den Niederlanden sind hohe Prävalenzen zwischen 60 und 80 % für *D. viviparus* in Milchviehherden beschrieben, gemessen mittels koproskopischer und serologischer Untersuchungen (Bloemhoff et al., 2015; Ploeger et al., 2012). Bennema et al. (2009) und Schunn et al. (2013) ermittelten deutlich niedrigere Prävalenzen zwischen 17 und 20 % basierend auf Antikörpertests unter Verwendung von Tankmilchproben in belgischen und deutschen Milchviehherden.

1.3.3 Infektionen mit dem großen Leberegel

Der große Leberegel *Fasciola hepatica* parasitiert bei Rindern in der Leber sowie in den Gallengängen und vermehrt sich auf der Weide über einen Zwischenwirt, die Zwergschlammschnecke *Galba truncatula*. Rinder infizieren sich oral durch die Aufnahme der infektiösen Zwischenstadien (Metazerkarien) auf der Weide oder über kontaminiertes Futter. Infektionen mit *F. hepatica* stellen ein hartnäckiges Problem in milchviehwirtschaftenden Betrieben mit Weidegang dar (Byrne et al., 2018; Selemetas et al., 2015). Wirtschaftliche Verluste ergeben sich durch verminderte Milchleistung, schlechtere Fruchtbarkeit sowie die verminderte Fleischqualität oder das Verwerfen von Lebern am Schlachthof. Die Einbußen in der durchschnittlichen Milchleistung liegen bei bis zu 4,2 kg/Kuh/Tag, basierend auf Studien zu Antikörperuntersuchungen in Tankmilchproben (Charlier et al., 2007a; Howell et al., 2015). Zudem wird ein Anstieg der *F. hepatica*-Herdenantikörpertiter mit einer Reduktion des Milchprotein-und Milchfettgehaltes um bis zu 0,09 % assoziiert (Charlier et al., 2007a). Knubben-Schweizer et al. (2010) und Schweizer et al. (2005) schätzten ökonomische Verluste in Höhe von 299 € pro Kuh im Zuge einer patenten *F. hepatica*-Infektion. In Irland liegen die Herdenprävalenzen bei über 80 %, erhoben auf Basis von Antikörpertests in Tankmilchproben (Byrne et al., 2018; Selemetas et al., 2015). In Deutschland wurden Herdenprävalenzen von bis zu 74 % beobachtet, wobei die höchsten Prävalenzen im Norden und Nordwesten Deutschlands gefunden wurden (Kuerpick et al., 2012). *F. hepatica*-Infektionen verlaufen bei adulten Rindern in der Regel chronisch mit lediglich milden klinischen Symptomen wie Abmagerung, Anämie und Leistungsminderung (Kaplan, 2001). Dennoch erwerben adulte Rinder keine vollständige protektive Immunität (Cawdery et al., 1977), so dass sie sich Jahr für Jahr erneut mit *F. hepatica* reinfizieren können (Kaplan, 2001). *F. hepatica* bewirkt eine Immunsuppression im Wirt (Molina-Hernández et al., 2015), was wiederum die Infektanfälligkeit für weitere infektiöse Erreger wie z.B. bakterielle Erkrankungen erhöht und damit sekundär ökonomische Verluste nach sich zieht (Claridge et al., 2012; Lucena et al., 2017).

1.4 In der Zucht verwendete Merkmale einer Endoparasitenresistenz

Die Eiausscheidung mit dem Kot oder Eizahl pro Gramm Kot (EpG), erfasst mittels koproskopischer Untersuchungen, stellt einen guten und üblicherweise verwendeten parasitologischen Indikator für die Zucht auf eine verbesserte Endoparasitenresistenz beim Wiederkäuer dar (Sonstegard und Gasbarre, 2001). Für das Merkmal EpG für GIN liegen die Erblichkeiten sowohl beim Schaf als auch beim Rind im moderaten Bereich (Morris und Amyes, 2012; Sonstegard und Gasbarre, 2001). Die Nutzung dieses Merkmals kann zu schnellem genetischen Fortschritt führen, wie dies eindrucksvoll in früheren Jahren beim Merinoschaf, aber auch beim Angus-Rind, gezeigt wurde (Gasbarre et al., 2002; Gray et al., 1992). Für Infektionen mit *D. viviparus* oder *F. hepatica* fanden koproskopische Merkmale wie die Ei- oder Larvenausscheidung im Kot bereits Anwendung in genetischen-statistischen Analysen (May et al., 2017). In der vorliegenden These werden ebenfalls Endoparasitenmerkmale auf Basis koproskopischer Untersuchungen in die Analysen einbezogen. Sensitivität und Spezifität der Koproskopie hängen maßgeblich vom verwendeten Verfahren sowie von epidemiologischen Faktoren des jeweiligen Erregers ab. Im Zuge von *F. hepatica*-Infektionen gelangen die Eier des in der Wirtsleber parasitierenden Erregers schubweise in die Galle, eine Eiausscheidung mit dem Kot erfolgt daher nicht regelmäßig. Dies kann einen Erregernachweis bei nur einmaliger Untersuchung erschweren und somit zu falsch negativen Ergebnissen führen. Weiterhin wurden in einigen Studien direkte parasitäre Parameter verwendet, wie Wurmlänge oder Fruchtbarkeit der Würmer, um eine Resistenz im Tier zu messen (Stear et al., 1997). Nachteil ist, dass diese Parameter nicht am lebenden Tier erfasst werden können und somit nur einmalige Messungen möglich sind. Neben der Eiausscheidung als einen Indikator patenter (aktuell bestehender) Infektionen im Wirt finden immunologische Indikatoren wie die Messung des Antikörpertiters in Serum oder Milch für die einzelnen parasitären Erreger Anwendung in genetischen Studien (Twomey et al., 2018, 2019). Die Nutzung von Antikörpertests für Resistenzstudien hat den Nachtweil, dass Antikörper über eine bestehende Infektion hinaus, je nach Helminthenart zwischen 7 Monaten und 2 Jahren, persistieren können (Castro et al., 2000; Fiedor et al., 2009). Bei alleiniger Verwendung von Antikörpertiteruntersuchungen kann daher nicht auf eine patente Infektion geschlossen werden. Zudem sind für kommerziell verfügbare serologische Tests Kreuzreaktionen mit Antikörpern anderer parasitärer Erreger beschrieben (Bennema et al., 2009). Weiterhin nutzten Twomey et al. (2016, 2019) auch direkte Merkmale einer patenten *F. hepatica*-Infektion, wie Leberschädigung (z.B. Kalzifizierung der Gallengänge) oder die Anwesenheit adulter Egel in den Gallengängen bei Schlachttieren. Beim Schaf finden weitere Merkmale wie z.B. der

Plasma-Pepsinogen oder Plasma-Albumin-Spiegel, die Messung des Anämiegrades mittels FAMACHA®-Score oder auch der Verschmutzungsgrad (Dag Score) sowie die Beurteilung der Kotkonsistenz in quantitativ-genetischen Studien Verwendung (Davies et al., 2005). Da alle der genannten Merkmale sehr zeitaufwändig und kostenintensiv in der Phänotypisierung sind, ist eine direkte Nutzung dieser Merkmale für die Zucht wenig praktikabel. Jedoch sind züchterische Verbesserungen in der Endoparasitenresistenz durch eine gezielte Selektion mittels zuvor identifizierter DNA-Marker möglich, auch wenn nur eine geringe Anzahl an Phänotypen zur Verfügung steht (Sonstegard and Gasbarre, 2001). Beim Rind werden das EpG und der Antikörpertiter derzeit als die verlässlichsten Marker für Resistenzschätzungen und für die Identifizierung von SNPs, die mit einer Endoparasitenresistenz assoziiert sind, angesehen (May et al., 2017; Sonstegard und Gasbarre, 2001; Twomey et al., 2019). Tabelle 1 liefert eine Übersicht zu den in genetischen Studien verwendeten Merkmalen für Infektionen mit GIN, *D. viviparus* und *F. hepatica* beim Rind.

Tabelle 1. In der Literatur angegebene Heritabilitäten (nach Publikationsjahr) für verschiedene Merkmale zur Erfassung von Infektionen mit gastrointestinalen Nematoden (GIN), *D. viviparus* und *F. hepatica*.

Referenz	Rasse	Merkmal	Heritabilität
GIN			
Barlow u. Piper 1985	Hereford	EpG	0,04 – 0,29
Leighton et al. 1989	Angus	EpG	0,29
Gasbarre et al. 1990	Angus	EpG	0,08 – 0,27
Mackinnon et al. 1991	Zebu-Kreuzungen	EpG	0,12 – 0,25
Kloosterman et al. 1992	HF	EpG	0,14 - 0,78
Gasbarre et al. 1993	Angus	Antikörpertiter	0,70 – 0,80
Zinsstag et al. 2000	West African N‘Dama	EpG	0,18
Burrow 2001	Austr. Belmont Red	EpG	0,36
Morris et al. 2003	Angus	EpG	0,32
		Antikörpertiter	0,06 – 0,40
Coppieters et al. 2009	HF	EpG	0,07 – 0,21
Morris u. Amyes 2012	Angus, Hereford	EpG	0,11 – 0,28

Referenz	Rasse	Merkmal	Heritabilität
GIN			
Passafaro et al. 2015	Nellore	EpG	0,06 – 0,33
May et al., 2017	HF, HF-Kreuzungen, DSN	EpG	0,05
Twomey et al. 2018	Holstein Friesian	Antikörpertiter	0,07
Rinderlungenwurm (*Dictyocaulus viviparus*)			
May et al., 2017	HF, HF-Kreuzungen, DSN	Larvenzahl im Kot	0,04
Mahmoud et al., 2018	HF	Diagnosedaten nach ZTGS	0,06*
Großer Leberegel (*Fasciola hepatica*)			
McClure et al. 2014	Milch/Fleischrinder	Adulte Egel in der Leber (binär)	0,15
Twomey et al. 2016	Milch/Fleischrinder	Antikörper Leberschädigung	0,09 – 0,10 0,01 – 0,03
May et al., 2017	HF, HF-Kreuzungen, DSN	Eizahl im Kot	0,33
Twomey et al. 2018	HF	Antikörpertiter	0,13

*SNP-basierte Heritabilität; DSN = Deutsches Schwarzbuntes Niederungsrind; EpG = Eizahl pro Gramm Kot; HF = Holstein Friesian; ZTGS = Zentraler Tiergesundheitsschlüssel nach Feucker u. Staufenbiel

1.5 Genetik der Endoparasitenresistenz beim Rind

Beim Rind werden züchterische Ansätze für eine verbesserte Resistenz gegen GIN-Infektionen seit Mitte der 80er Jahre verfolgt. Die Ergebnisse derartiger Studien zeigten rassespezifische Variationen für das Merkmal GIN-Eiausscheidung (Barlow und Piper 1985; Leighton et al., 1989; Oliveira et al., 2009; Suarez 1990, 1995). Barlow und Piper (1985) und Kloosterman et al. (1978) wiesen phänotypische Varianzen für verschiedene Bullenväter innerhalb Kälber der Rassen HF, Hereford, Simmental und Brahman für die GIN-Eiausscheidung nach. In den folgenden Jahren wurden weitere Vergleichsstudien zwischen reinrassigen Rindern und

Kreuzungstieren für verschiedene Merkmale einer GIN-Resistenz publiziert, wobei sich die Kreuzungszuchten nicht in allen Studien als überlegen erwiesen (May et al., 2017; Oliveira et al., 2009; Suarez et al., 1990).

Zunehmende Anthelminthika-Resistenzen von Endoparasiten stellen ein globales Problem in der Rinderhaltung dar (Geurden et al., 2015; Waghorn et al., 2016) und implizieren die Evaluierung züchterischer Selektionsstrategien für eine verbesserte Endoparasitenresistenz im Wirt. Beim Rind liegen die Heritabilitäten für GIN-Infektionen oder für einzelne GIN-Spezies zwischen 0,04 und 0,80, gemessen anhand der Merkmale Eiausscheidung oder Antikörpertiter (Tabelle 1). In den meisten Studien liegen die Heritabilitäten im moderaten Bereich zwischen 0,30 und 0,40 (Tabelle 1). In Milchrindern der Rasse HF liegen die Heritabilitäten zwischen 0,05 und 0,21 (Coppieters et al., 2009; May et al., 2017; Twomey et al., 2018). May et al. (2017) schätzten eine niedrige Heritabilität von 0,05 für das Merkmal „*D. viviparus*-Larvenausscheidung". Die genetischen Korrelationen zwischen den beiden Resistenzmerkmalen für GIN und *D. viviparus* zeigten überwiegend negative genetische Korrelationen mit den Milchproduktionsmerkmalen Milch-kg, Milchprotein- und Fettgehalt sowie dem Fett-Eiweiß-Quotient (FEQ). Mahmoud et al. (2018) schätzten auf Basis von SNP-Daten eine Heritabilität von 0,06 für die von Landwirten erfasste Diagnose „*D. viviparus*-Infektion". McClure et al. (2014) und Twomey et al. (2016, 2018) publizierten Heritabilitäten zwischen 0,01 und 0,15 für Merkmale einer „*F. hepatica*-Resistenz" (IgG-Titer, Leberschädigung oder Präsenz adulter Egel in der Leber) in verschiedenen Rassen von Milch- und Fleischrindern in Irland. May et al. (2017) schätzten für das Merkmal *F. hepatica*-Eiausscheidung eine höhere Heritabilität von 0,33. Die Autoren schätzten überwiegend moderate negative genetische Korrelationen zwischen dem Merkmal *F. hepatica*-Eiausscheidung und den Testtagsmerkmalen Milch-kg und Milchproteingehalt. Twomey et al. (2018) schätzten niedrige genetische Korrelationen von -0,14 bis 0,10 zwischen *O. ostertagi*- und *F. hepatica*- Antikörpertitern und Milchproduktionsmerkmalen.

Die erste Studie zur Identifizierung von mit einer Endoparasitenresistenz assoziierten Quantitative Trait Loci (QTL) im Rind basierte auf einem Datensatz von 768 selektiv genotypisierten und mit GIN natürlich infizierten HF Kühen (Coppieters et al., 2009). In dieser Studie wurden QTL auf dem bovinen Chromosom (BTA) 9 und 19 für das Merkmal GIN-Eiausscheidung gefunden. Die Autoren postulierten zudem, dass das *ITGAE* Gen auf BTA 19 mit einer Immunreaktion im Wirt im Zuge von GIN-Infektionen in Verbindung stehen könnte. Intensive Studien zur Identifizierung von QTL, welche mit einer GIN-Resistenz assoziiert sind, wurden in Angus-Rindern durchgeführt (Kim et al., 2014, 2015; Liu et al., 2011). Kim et al.

(2014) assoziierten QTL auf BTA 4, 8, 12 and 17 mit dem Resistenzmerkmal „GIN-Eiausscheidung“ in einer Angus-Population basierend auf Mikrosatelliten-Markern. Twomey et al. (2019) führten eine genomweite Assoziationsstudie (GWAS) für Merkmale einer *F. hepatica*-Resistenz (Leberschädigung nach patenter Infektion und Antikörpertiter) basierend auf imputeten Genotypdaten irischer Milch- und Fleischrinder durch. Die Autoren identifizierten genomische Regionen auf BTA 1, 8, 11, 16, 17 and 18, welche mit dem Merkmal „Leberschädigung infolge patenter *F. hepatica*-Infektion“ assoziiert waren sowie auf BTA 6, 14, 15 für die Höhe der anti-*F. hepatica*-Antikörpertiter. Zudem fanden die Autoren ein QTL auf BTA 7, welches mit beiden Infektionsmerkmalen assoziiert war. Desweiteren beschrieben Twomey et al. (2019) das *RHOH* Gen als potentielles Kandidatengen, welches in die Aktivierung von Mastzellen und damit in die Antikörperbildung während *F. hepatica*-Infektionen involviert sein könnte, da ein Anstieg an Mastzellen im Wirt der Immunabwehr dienen.

1.6 Genomische Ansätze zur Zucht auf Endoparasitenresistenz beim Deutschen Schwarzbunten Niederungsrind

Die Kapitel 3 und 4 der vorliegenden Arbeit fokussieren auf das Deutsche Schwarzbunte Niederungsrind (DSN) als eine vom Aussterben bedrohte Zweinutzungsrasse mit kleiner Populationsgröße. Das DSN ist eine der Gründerrassen der heutigen HF Population und gilt als besonders gut adaptiert an Weideproduktionsysteme (Mügge, 1999). Al-Kanaan (2016) und Jaeger et al. (2016) konnten zeigen, dass das DSN besonders gut an das Umweltsystem „Weide“ angepasst ist und eine gute Toleranz gegenüber Hitzestress sowie eine gute Krankheitsresistenz besitzt. Eine Untersuchung der besonderen charakteristischen Merkmale des DSN, welche mit einer verbesserten Krankheitsresistenz und Robustheit im Produktionssystem Weide verbunden sind, sind daher wichtig für den Erhalt der Population. Derartige Untersuchungen beinhalten insbesondere genomische Analysen, welche für zukünftige, speziell auf das DSN angepasste Zuchtziele oder in der Weiterentwicklung von SNP-Chips für die genomische Selektion der Rasse genutzt werden können.

In großen Milchrindpopulationen wie HF ist die genomische Selektion inzwischen zu einem viel genutzten züchterischen Instrument geworden, um einen schnellen Zuchtfortschritt in ökonomisch relevanten Merkmalen zu realisieren (Weller et al., 2017). In diesem Zuge wurde auch die Inkludierung direkter Gesundheitsdaten (vom Landwirt erfasste Diagnosen) und Indikatormerkmale in der genomischen Selektion für die kommerziellen Rassen zügig vorangetrieben und bietet neue Möglichkeiten zur Verbesserung der Krankheitsresistenz (Calus

et al., 2013; Chesnais et al., 2016). Für gefährdete, kleine Populationen schreitet die derzeitige Entwicklung in der genomischen Selektion hingegen nur sehr langsam voran. Hauptgrund ist, dass keine genügend große Referenzpopulation vorliegt um genaue und zuverlässige Zuchtwerte schätzen zu können (Pryce und Daetwyler, 2012; Schöpke und Swalve, 2016). Zudem ist die Anzahl aussagekräftiger Phänotypen in kleinen Populationen wie dem DSN häufig zu gering ist. Die Aufrechterhaltung der genetischen Diversität und der Erhalt kleiner, gefährdeter Populationen trägt jedoch maßgeblich zur Verbesserung von Robustheit und Krankheitsresistenz in der Rinderpopulation bei (Biscarini et al., 2015; Bishop, 2012). Daher war es ein Ziel der vorliegenden Arbeit, genomische und molekulargenetische Analysen zur Endoparasitenresistenz (Kapitel 3 und 4) am Beispiel des DSN durchzuführen.

Ziele der Arbeit

Die vorliegende Arbeit beschäftigt sich mit züchterischen Selektionsstrategien und Methodiken für eine Verbesserung der Robustheit, Resistenz, Toleranz und Widerstandsfähigkeit bei Milchkühen. Anhand des Beispiels endoparasitärer Infektionen werden verschiedenste statistische Methoden angewandt, um zugrunde liegende genetische, immunologische und physiologische Mechanismen einer Resistenz bei Milchkühen zu identifizieren.
Die Ziele der Arbeit gliedern sich wie folgt:

1. Kapitel 2 befasst sich mit Phänotypisierungstrategien und deren historischer Entwicklung sowie mit der Schätzung quantitativ-genetischer Parameter für Merkmale, die mit einer Robustheit bei Milchkühen assoziiert sind. Der Fokus liegt dabei insbesondere auf Merkmalen, die als Biomarker für infektiöse, durch bakterielle und parasitäre Erreger induzierte Erkrankungen genutzt werden.
2. Ziel von Kapitel 3 ist die Durchführung einer GWAS zur Identifizierung von SNPs, potentiellen Kandidatengenen und (immunologischen) Pathways, die mit einer Endoparasitenresistenz assoziiert sind. Dabei wurde auf Infektionen mit GIN, *F. hepatica* und *D. viviparus* in der vom Aussterben bedrohten Rinderpopulation DSN mit kleiner Populationsgröße fokussiert. Die einbezogenen Phänotypen für Endoparasitenmerkmale basierten auf einem größeren Datensatz schwarzbunter Milchkühe. Weiteres Ziel war die Schätzung von SNP-Effekt Korrelationen zwischen den Endoparasitenmerkmalen und den Testtagsmerkmalen Milch-kg und transformierte somatische Zellzahl (SCS) für identifizierte chromosomale Abschnitte, die eine Endoparasitenresistenz beeinflussen.

3. Kapitel 3 fokussiert auf die Identifizierung von Polymorphismen in Exon 9 und flankierenden intronischen Sequenzen des Activated leukocyte cell adhesion molecule (*ALCAM*) Gens auf dem bovinen Chromosom 1 in DSN. Weiterhin sollten Allelsubstitutionseffekte und Dominanzeffekte für die identifizierten SNPs im *ALCAM* Gen für Merkmale einer Endoparasitenresistenz, für Testtagsmerkmale und weitere Merkmale einer Krankheitsresistenz und Robustheit unter Verwendung logistischer Regressionsmodelle geschätzt werden.
4. Ziel von Kapitel 4 ist die Erhebung der *F. hepatica*-Seroprävalenz in den Jahren 2017 und 2018 in norddeutschen Milchviehherden sowie die Schätzung von Assoziationen zwischen dem *F. hepatica*-Antikörpertiter in Tankmilchproben und Milchleistungsmerkmalen sowie der somatischen Zellzahl in der Milch auf Herdenebene. Weiterhin sollte der Einfluss der *F. hepatica*-Antikörpertiter in der Tankmilch auf die in der FTIR Spektrometrie abgeleiteten Milchketonkörper-Werte BHB und Aceton untersucht werden.

Referenzen

Agneessens J, Clearebout E, Dorny P, Borgsteede FHM, Vercruysse J. 2000. Nematode parasitism in adult dairy cows in Belgium. Veterinary Parasitology 90:83-92.

Ahn BS, Jeon BS, Kwon EG, Ajmal Khan M, Kim HS, Ju JC, Kim NS. 2006. Estimation of genetic parameters for daily milk yield, somatic cell score, milk urea nitrose, blood glucose and immunoglobulin in Holsteins. Asian-Australian Journal of Animal Science 19:1252-1256.

Al-Kanaan A. 2016. Heat stress response for physiological traits in dairy and dual-purpose cattle populations on phenotypic and genetic scales. Universität Kassel, Dissertation.

Barlow R, Piper LR. 1985. Genetic analyses of nematode egg counts in Hereford and crossbred Hereford cattle in the subtropics of New South Wales. Livestock Production Science 12: 79-84.

Bellet C, Green MJ, Vickers M, Forbes A, Berrx E, Kaler J. 2016. *Ostertagia spp.*, rumen fluke and liver fluke single- and poly-infections in cattle: An abattoir study of prevalence and production impacts in England and Wales. Preventive Veterinary Medicine 132:98-106.

Bishop SC. 2012. A consideration of resistance and tolerance for ruminant nematode infections. Frontiers in Genetics 3:168.

Bennema S, Vercruysse J, Claerebout E, Schnieder T, Strube C, Ducheyne E. 2009. The use of bulk-tank milk ELISAs to assess the spatial distribution of *Fasciola hepatica*, *Ostertagia ostertagi* and *Dictyocaulus viviparus* in dairy cattle in Flanders (Belgium). Veterinary Parasitology 165:51–57.

Biscarini F, Nicolazzi EL, Stella A, Boettcher PJ, Gandini G. 2015. Challenges and opportunities in genetic improvement of local livestock breeds. Frontiers in Genetics 6:33.

Bloemhoff Y, Forbes A, Good B, Morgan E, Mulcahy G, Strube C, Sayers R. 2015. Prevalence and seasonality of bulk tank milk antibodies against *Dictyocaulus viviparus* and *Ostertagia ostertagi* in Irish pasture-based dairy herds. Veterinary Parasitology 209:108-116.

Burrow HM. 2001. Variances and covariances between productive and adaptive traits and temperament in a composite breed of tropical beef cattle. Livestock Production Science 70:213-233.

Byrne AW, Graham J, McConville J, Milne G, McDowell S, Hanna REB, Guelbenzu-Gonzalo M. 2018. Seasonal variation of *Fasicola hepatica* antibodies in dairy herds in Northern Ireland measured by bulk tank milk ELISA. Parasitology Research 117:2725-2733.

Castro E, Freyre A, Hernandez Z. 2000. Serological responses after treatment and during natural re-infection with *Fasciola hepatica*, as measured with a dot-ELISA system. Veterinary Parasitology 90:201-208.

Cawdery MJH, Strickland KL, Conway A, Crowe PJ. 1977. Production effects of liver fluke in cattle Part 1. Effects of infection on live weight gain, feed intake and food conversion efficiency in beef cattle. British Veterinary Journal 133:145-159.

Charlier J, Duchateau L, Clearebout E, Williams E, Vercruysse J. 2007a. Associations between anti-*Fasciola hepatica* antibody levels in bulk-tank milk samples and production parameters in dairy herds. Veterinary Medicine 78:57-66.

Charlier J, Duchateau L, Clearebout E, Vercruysse J. 2007b. Predicting milk-production responses after an autumn treatment of pastured dairy herds with eprinomectin. Veterinary Parasitology 143:322-328.

Charlier J, De Cat A, Forbes A, Vercruysse J. 2009. Measurement of antibodies to gastrointestinal nematodes and liver fluke in meat juice of beef cattle and associations with carcass parameters. Veterinary Parasitology 166:235-240.

Chesnais JP, Cooper TA, Wiggans GR, Sargolzaei M, Pryce JE, Miglior F. 2016. Using genomics to enhance selection of novel traits in North American dairy cattle. Journal of Dairy Science 99:2413-2427.

Charlier J, Ghebretinsae A, Meyns T, Czaplicki G, Vercruysse J, Clearebout E. 2016. Antibodies against *Dictyocaulus viviparus* major sperm protein in bulk tank milk: Association with clinical appearance, herd management and milk production. Veterinary Parasitology 232:36–42.

Calus MPL, Berry DP, Banos G, de Haas Y, Veerkamp RF. 2013. Genomic selection: the option for new robustness traits? Advances in Animal Biosciences 4:618-625.

Calus MPL, Veerkamp RF. 2003. Estimation of environmental sensitivity of genetic merit for milk production traits using random regression model. Journal of Dairy Science 86:3756-3764.

Claridge J, Diggle P, McCann CM, Mulcahy G, Flynn R, Mcnair J, Strain S, Welsh M, Baylis M, Williams DJL. 2012. *Fasciola hepatica* is associated with the failure to detect bovine tuberculosis in dairy cattle. Nature Communications 3:853.

Coppieters W, Mes THM, Druet T, Farnir F, Tamma N, Schrooten C, Cornelissen AWCA, Georges M, Ploeger HW. 2009. Mapping QTL influencing gastrointestinal nematode burden in Dutch Holstein-Friesian dairy cattle. BMC Genomics 10:96.

Davies G, Stear MJ, Bishop SC. 2005. Genetic relationships between indicator traits and nematode infection levels in 6-month-old lambs. Animal Science 80:143-150.

Dank M, Holzhauer M, Veldhuis A, Frankena K. 2015. Association between *Dictyocaulus viviparus* status and milk production parameters in Dutch dairy herds. Journal of Dairy Science 98:7741–7747.

Detilleux JC, Koehler KJ, Freeman AE, Kehrli ME, Jr. Kelley DH. 1994. Immunological parameters of periparturient Holstein cattle: genetic variation. Journal of Dairy Science 77:2640-2650.

Haile-Mariam D, Carrick MJ, Goddard ME. 2008. Genotype by environment interaction for fertility, survival, and milk production traits in Australian dairy cattle. Journal of Dairy Science 91:4840-4853.

Egger-Danner C, Cole JB, Pryce JE, Gengler N, Heringstad B, Bradley A, Stock KF. 2015. Invited review: Overview of new traits and phenotyping strategies in dairy cattle with a focus on functional traits. Animal 9:191-207.

Fiedor C, Strube C, Forbes A, Buschbaum S, Klewer AM, von Samson-Himmelstjerna G. 2009. Evaluation of a milk ELISA for the serodiagnosis of *Dictyocaulus viviparus* in dairy cows. Veterinary Parasitology 166:255–261.

Fikse WF, Rekaya R, Weigel KA. 2003. Assessment of environmental descriptors for studying genotype by environment interaction. Livestock Production Science 82:223-231.

Gauly M, Bollwein H, Breves G, Brügemann K. 2013. Future consequences and challenges for dairy cow production systems arising from climate change in Central Europe - a review. Animal 7:843-859.

GasbarrE LC, Leighton EA, Davies CJ. 1990. Genetic control of immunity to gastrointestinal nematodes of cattle. Veterinary Parasitology 37:257-272.

Gasbarre LC, Leighton EA, Davies CJ. 1993. Influence of host genetics upon antibody responses against gastrointestinal nematode infections in cattle. Veterinary Parasitology 46:81-91.

Gasbarre LC, Sonstegard T, van Tassell CP, Padilha T. 2002. Detection of QTL affecting parasite resistance in a selected herd of Angus cattle. Proceedings of the 7th World Congress on Genetics Applied to Livestock Production, Montpellier, France, p. 19-23.

Geurden T, Chartier C, Franke J, Di Regalbono AF, Traversa D, von Samson-Himmelstjerna G, Demeler J, Vanimisetti HB, Batram DJ, Denwood MJ. 2015. Anthelmintic resistance to ivermectin and moxidectin in gastrointestinal nematodes of cattle in Europe. International Journal for Parasitology: Drugs and Drug Resistance 5:163-171.

Gray GD, Barger IA, Le Jambre LF, Douch PGC. 1992. Parasitological and immunological responses of genetically resistant Merino sheep on pastures contaminated with parasitic nematodes. International Journal for Parasitology 22:417-425.

Heriazon A, Quinton M, Miglior F, Leslie KE, Sears W, Mallard BA. 2013. Phenotypic and genetic parameters of antibody and dalyed-type hypersensitivity responses of lactating Holstein cows. Veterinary Immunology and Immunopathology 154:83-92.

Holzhauer M, van Schaik G, Saatkamp HW, Ploeger HW. 2011. Lungworm outbreaks in adult dairy cows: estimating economic losses and lessons to be learned. Veterinary Record 196:494.

Howell A, Baylis M, Smith R, Pinchbeck G, Williams D. 2015. Epidemiology and impact of *Fasciola hepatica* exposure in high-yielding dairy herds. Preventive Veterinary Medicine 121:41-48.

Jaeger M, Brügemann K, König S. 2016. Genetic relationships and trait comparison between and within lines of local dual-purpose cattle. 67th Annual meeting of the European Association for Animal Production, Belfast, Irland.

Kaplan RM. 2001. *Fasciola hepatica*: A review of the economic impact in cattle and considerations for control. Veterinary Therapeutics 2:40-50.

Kim ES, Sonstegard TS, Silva MV, Gasbarre LC, van Tassell CP. 2014. Identification of quantitative trait loci affecting gastrointestinal parasite resistance in an experimental Angus population. Animal Genetics 45:117-121.

Kim ES, Sonstegard TS, Silva MV, Gasbarre LC, van Tassell CP. 2015. Genome-wide scan of gastrointestinal nematode resistance in closed Angus population selected for minimized influence of MHC. PLoS One 10:e0119380.

Kloosterman A, Albers GAA, van den Brink R. 1978. Genetic variation among calves in resistance to nematode parasites. Veterinary Parasitololy 4:353-368.

Knubben-Schweizer G, Ruegg S, Torgerson PR, Rapsch C, Grimm F, Hassig M, Deplazes P, Braun U. 2010. Control of bovine fasciolosis in dairy cattle in Switzerland with emphasis on pasture management. Veterinary Journal 186:188-191.

Kuerpick B, Schnieder T, Strube C. 2012. Seasonal pattern of *Fasciola hepatica* antibodies in dairy herds in Northern Germany. Parasitology Research 111:1085-1092.

Leighton EA, Murrell KD, Gasbarre LC. 1989. Evidence for genetic control of nematode egg-shedding rates in calves. Journal of Parasitology 75:498-504.

Liu GE, Brown, T, Hebert DA, Cardone MF, Hou Y, Choudhary RK, et al. 2011. Intitial analysis of copy number variations in cattle selected for reistance or susceptibility to intestinal nematodes. Mammalian Genome 22:111-121.

Lucena AN, Cuartero L, Mulcahy G, Zintl A. 2017. The immunomodulatory effects of co-infection with *Fasciola hepatica*: From bovine tuberculosis to Johne's disease. The Veterinary Journal 222:9-16.

Mackinnon MJ, Meyer K, Hetzel DJS. 1991. Genetic variation and covariation for growth, parasite resistance and heat tolerance in tropical cattle. Livestock Production Science 27:105-122.

May K, Brügemann K, Yin T, Scheper C, Strube C, König S. 2017. Genetic line comparisons and genetic parameters for endoparasite infections and test-day milk production traits. Journal of Dairy Science 100:7330-7344.

May K, Brügemann K, König S, Strube C. 2018. The effect of patent *Dictyocaulus viviparus* (re)infections on individual milk yield and milk quality in pastured dairy cows and correlation with clinical signs. Parasites & Vectors 11:24.

McClure J, Cromie AR, Sayers R, Graham D, Byrne N, Berry DP. 2014. Genetics of susceptibility to bovine diarrhea virus, bovine herpesvirus type 1 and *Fasciola hepatica* in cattle. In: 10. Kongress Genetik für die Tierproduktion Vancouver, S. 542-545.

Mehmood K, Zhang H, Sabir AJ, Abbas RZ, Ijaz M, Durrani AZ, et al. 2018. A review on epidemiology, global prevalence and economical losses of fasciolosis in ruminants. Microbial Pathogenesis 109:253-262.

Molina-Hernández V, Mulcahy G, Pérez J, Martínez-Moreno Á, Donnelly S, O´Neill SM, Dalton JP, Cwiklinski K. 2015. *Fasciola hepatica*: We may not be there yet but we´re on the right road. Veterinary Parasitology 208:101-111.

Morris CA, Green RS, Cullen NG, Hickey SM. 2003. Genetic and phenotypic relationships among faecal egg count, anti-nematode antibody level and live weight in Angus cattle. Animal Science 76:167-174.

Morris CA, Amyes NC. 2012. Heritability and repeatability of resistance to nematode parasites in commercial beef cattle. Proceedings of the New Zealand Society of Animal Production 72:236-239.

Mügge B, Lutz WE, Südbeck H, Zelfel S. 1999. Deutsche Holsteins: Die Geschichte einer Zucht. 1. Aufl. Verlag Eugen Ulmer, Stuttgart, S. 1-247.

Neuenschwander TF-O, Miglior F, Jamrozik J, Berke O, Kelton DF, Schaeffer LR. 2012. Genetic parameters for producer-recorded health data in Canadian Holstein cattle. Animal 6:571-578.

Nødtvedt A, Dohoo I, Sanchez J, Conboy G, DesCôteaux L, Keefe G. 2002. Increase in milk yield following eprinomectin treatment at calving in pastured dairy cattle. Veterinary Parasitology 105:191-206.

Oliveira MCS, Alencar MM, Chagas ACS, Giglioti R, Oliveira NH. 2009. Gastrointestinal nematode infection in beef cattle of different genetic groups in Brazil. Veterinary Parasitology 166:249-254.

Passafaro TL, Carrera JPB, dos Santos LL, Raidan FSS, dos Santos DCC, Cardoso EP, Leite RC, Toral FLB. 2015. Genetic analysis of resistance to ticks, gastrointestinal nematodes and *Eimeria* spp. in Nellore cattle. Veterinary Parasitology 210:224-234.

Perri AF, Mejía ME, Licoff N, Lazaro L, Miglierina M, Ornstein A, Becu-Villalobos D, Lacau-Mengido IM. 2011. Gastrointestinal parasites presence during peripartum decreases total milk production in grazing dairy Holstein cows. Veterinary Parasitology 178:311-318.

Ploeger HW. 2002. *Dictyocaulus viviparus*: Re-emerging or never been away? Trends in Parasitology 18:329–332.

Ploeger HW, Verbeek PC, Dekkers CW, Strube C, Van Engelen E, Uiterwijk M. 2012. The value of a bulk-tank milk ELISA and individual serological and faecal examination for diagnosing (sub)clinical *Dictyocaulus viviparus* infection in dairy cows. Veterinary Parasitology 184:168–179.

Pryce JE, Daetwyler HD. 2012. Designing dairy cattle breeding schemes under genomic selection: a review of international research. Animal Production Science 52:107-114.

Pryce JE, Gaddis KP, Koeck A, Bastin C, Adessayed M, Gengler N, Miglior F, Heringstad B, Egger-Danner C, Stock K. 2016. Invited review: Opportunities for genetic improvement of metabolic diseases. Journal of Dairy Science 99:6855-6873.

Rashidi H. 2016. Breeding against infectious diseases in animals. Dissertation, Wageningen.

Rauw WM, Gomez-Raja L. 2015. Genotype by environment interaction and breeding for robustness in livestock. Frontiers in Genetics 6:310.

Robertson A. 1959. The sampling variance of the genetic correlation coefficient. Biometrics 15:469-485.

Schöpke K, Swalve HH. 2016. Review: Opportunities and challenges for small populations of dairy cattle in the era of genomics. Animal 10:1050-1060.

Schunn AM, Forbes A, Schnieder T, Strube C. 2012. Validation of a *Dictyocaulus viviparus* MSP-ELISA and cut-off adjustment in a one-year longitudinal field study in dairy cattle herds. Veterinary Parasitology 189:291–298.

Schunn AM, Conraths FJ, Staubach C, Fröhlich A, Forbes A, Schnieder T. 2013. Lungworm infections in German dairy cattle herds - seroprevalence and GIS-supported risk factor analysis. PloS One 8:e74429.

Schweizer G, Braun U, Deplazes P, Torgerson PR. 2005. Estimating the financial losses due to bovine fasciolosis in Switzerland. Veterinary Record 157:188-193.

Selemetas N, Ducheyne E, Phelan P, O'Kiely P, Hendrickx G, de Waal T. 2015. Spatial analysis and risk mapping of *Fasciola hepatica* infection in dairy herds in Ireland. Geospatial Health 9:281-291.

Singh J, Murray RD, Mshelia G, Woldehiwet Z. 2008. The immune status of the bovine uterus during the postpartum period. The Veterinary Journal 175:301-309.

Sonstegard TS, Gasbarre LC. 2001. Genomic tools to improve parasite resistance. Veterinary Parasitology 101:387-403.

Soyeurt H, Dehareng F, Romnée JM, Gengler N, Dardenne P. 2012. Genetics of the mineral contents in bovine milk predicted by mid-infrared spectrometry. 63rd Annual Meeting of the Association of European Animal Production, 27 August, Bratislava, Slovakia. Retrieved 08 March 2018, from https://orbi.uliege.be/bitstream/2268/129984/1/Session 03_Soyeurt.pdf.

Stear MJ, Bairden K, Duncan JL, Holmes PH, McKellar QA, Park M, Strain S, Murray M, Bishop SC, Gettinby G. 1997. How hosts control worms. Nature 389:27.

Streit M, Reinhardt F, Thaller G, Bennewitz J. 2012. Reaction norms and genotype-by-environment interaction in the German Holstein dairy cattle. Journal Animal Breeding Genetics 129:380–389.

Suarez VH, Ciminari OE, Bedotti DO, Busetti MR, Bello EM. 1990. Epidemiology, effects and control of nematode infections on Zebu Crossbred, Hereford and Hereford x Brahman calves of Argentina´s western pampas. Veterinary Parasitology 35:79-91.

Suarez VH, Busetto MR, Lorenzo RM. 1995. Comparative effects of nematode infection on *Bos taurus* and *Bos indicus* crossbred calves grazing on Argentina's Western Pampas. Veterinary Parasitology 58:263-271.

Thompson-Crispi KA, Sewalem A, Miglior F, Mallard BA. 2012. Genetic parameters of adaptive immune response traits in Canadian Holsteins. Journal of Dairy Science 95:401-409.

Thompson-Crispi KA, Miglior F, Mallard BA. 2013. Genetic parameters for natural antibodies and associations with specific antibody and mastitis in Canadian Holsteins. Journal of Dairy Science 96:3965-3972.

Twomey AJ, Sayers RG, Carroll RI, Byre N, O'Brien E, Doherty ML, McClure JC, Graham DA, Berry DP. 2016. Genetic parameters for both a liver damage phenotype caused by *Fasciola hepatica* and antibody response to *Fasciola hepatica* phenotype in dairy and beef cattle. Journal of Animal Science 94:4109-4119.

Twomey AJ, Carroll RI, Doherty ML, Byrne N, Graham DA, Sayers RG, Blom A, Berry DP. 2018. Genetic correlations between endo-parasite phenotypes and economically important traits in dairy and beef cattle. Journal of Animal Science 96:407-421.

Twomey AJ, Berry DP, Evans RD, Doherty ML, Graham DA, Purfield DC. 2019. Genome-wide association study of endo-parasite phenotypes using imputed whole-genome sequence data in dairy and beef cattle. Genetic Selection Evolution 51:15.

Van Haelst YNT, Beeckman A, van Knegsel ATM, Fievez V. 2008. Short Communication: Elevated concentrations of oleic acid and long-chain fatty acids in milk fat of multiparous subclinical ketotic cows. Journal of Dairy Science 91:4683-4686.

Vercruysse J, Charlier J, Van Dijk J, Morgan ER, Geary T, von Samson-Himmelstjerna, Clearebout E. 2018. Control of helminth ruminant infections by 2030. Parasitology 145:1655-1664.

Waghorn TS, Miller CM, Leathwick DM. 2016. Confirmation of ivermectin resistance in *Ostertagia ostertagi* in cattle in New Zealand. Veterinary Parasitology 229:139-143.

Wapenaar W, Barkema HW, Eysker M, O'Handley RM. 2007. An outbreak of dictyocaulosis in lactating cows on a dairy farm. Journal of the American Veterinary Medical Association 231:1715–1718.

Weller JI, Ezra E, Ron M. 2017. Invited review: A perspective on the future of genomic selection in dairy cattle. Journal of Dairy Science 100:8633-8644.

Woolley H. 1997. The economic impact of "husk" in dairy cattle. Cattle Practice 5:315-318.

Zinsstag J, Ankers P, M, Njie M, Smith T, Pandey VS, Pfister K, Tanner M. 2000. Heritability of gastrointestinal nematode faecal egg counts in West African village N´Dama cattle and its relation to age. Veterinary Parasitology 89:71-78.

Zwald NR, Weigel KA, Chang YM, Welper RD, Clay JS. 2004. Genetic selection for health traits using producer-recorded data I. Incidence rates, heritability estimates, and sire breeding values. Journal of Dairy Science 87:4287-4294.

KAPITEL 2

Phenotyping strategies and quantitative-genetic background of resistance, tolerance and resilience associated traits in dairy cattle

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Abstract

In dairy cattle, resistance, tolerance and resilience refer to the adaptation ability to a broad range of environmental conditions, implying stable performances (e.g., production level, fertility status) independent from disease or infection pressure. All three mechanisms resistance, tolerance and resilience contribute to overall robustness, implying the evaluation of phenotyping and breeding strategies for improved robustness in dairy cattle populations. Classically, breeding approaches on improved robustness rely on simple production traits, in combination with detailed environmental descriptors and enhanced statistical modelling to infer possible genotype by environment (G x E) interactions. In this regard, innovative environmental descriptors were heat stress indicators, and statistical modelling focussed on random regression or reacton norm methodology. A robust animal has high breeding values over a broad spectra of environmental levels. During the last years, direct health traits were included into selection indices, implying advances in genetic evaluations for traits being linked to resistance or tolerance against infectious and non-infectious diseases. Up to now, genetic evaluation for health traits is primarily based on subjectively measured producer-recorded data, with disease trait heritabilities in a low to moderate range. Thus, it is imperative to identify objectively measurable phenotypes as suitable biomarkers. New technologies (e.g., mid infrared spectrometry (MIR)) offer possibilities to determine potential biomarkers via laboratory analyses. Novel biomarkers include measurable physiological traits (e.g., serum metabolites, hormone levels) as indicators for a current infection, or the host's reaction to environmental stressors. The rumen microbiome composition is proposed as a biomarker to detect interactions between host genotype and environmental effects. The understanding of host genetic variation in disease resistance and individual expression of robustness encourages analyses on the underlying immune response system. Recent advances have been made in order to infer the genetic background of immune response (IR) traits and cows immunological competence in relation to functional and production traits. Thus, a last aspect of this review addresses the genetic background and current state of genetic control for resistance to economically relevant infectious and non-infectious dairy cattle diseases by considering immune-related factors.

Implications

Resistance, tolerance and resilience are components of overall robustness, reflecting the host's adaptation to environmental stressors and its interaction with disease mediated factors. Increasing environmental challenges, e.g., due to global climate change, lead to a higher infection pressure by various pathogens. Metabolic stress, especially in high yielding cows, is

a major reason for increased incidence rates for non-infectious diseases. Environmental alterations can impair the bovine immune system, having a direct or indirect effect on disease susceptibility. There is an urgent need to identify suitable phenotypes and novel markers related to immunological mechanisms, to improve overall immune response via optimized breeding strategies.

Introduction

Breeding on robustness via selection for enhanced disease resistance is of increasing importance in the dairy livestock industry worldwide (Calus et al., 2013). Classical breeding goals focused on improving milk or protein yield, but simultaneously neglected functional traits or health (Egger-Danner et al., 2015). As an antagonistic effect, long-term selection on increased productivity was associated with physiological and immunological imbalances, especially in the early lactation period. Immunological imbalances might stimulate the susceptibility to environmental influences, thus causing a decline in robustness (Rauw and Gomez-Raya, 2015). Negative side effects due to intensive selection on milk yield, the increasing value of animal welfare, ethical aspects, and profit maximization via reduced medical treatments (e.g., antibiotics, anthelmintics) are the main arguments supporting breeding strategies on improved disease resistance. Moreover, environmental challenges, especially the heterogeneity of climatic impact, are major driven forces towards breeding of robust dairy cows. Breeding on robustness implies the identification of animals with a quite high and constant production level for a wide range of climatic conditions and production systems, accompanied with a high level of animal welfare (Rauw and Gomez-Raja, 2015). The inclusion of functional traits into overall dairy cattle breeding goals was a first step towards breeding on robustness (Martin et al., 2018), implying rapid advances in genetic evaluations for traits being linked to disease resistance or tolerance.

The first section of this review addresses classical breeding approaches in dairy catte in order to improve robustness via modelling reactions to environmental alterations. Relevant genotype by environment (G x E) interactions in dairy cattle (e.g., environmental sensitivity, reaction norms, phenotypic plasticity) will be discussed, considering new characteristics of disease associated factors (e.g., herd prevalence, pathogen load) as environmental descriptors. The inclusion of binary subjectively recorded producer health traits into overall breeding goals is a next step to improve disease resistance. Third, suitable novel biomarkers for resistance, tolerance and resilience-associated traits from laboratory analyses (e.g., enzymes, milk fatty acids), will be reviewed (e.g., Pryce et al., 2016; Pieper et al., 2016). Advances in laboratory

analysis also allow deeper insights into immune genetics, aiming on the identification of suitable and practicable immune response (IR) traits (e.g., Thompson-Crispi et al., 2012). In this context, we will infer associations between IR traits and relevant production and health traits, in order to bridging the gap between immunity and selection for robustness.

Breeding approaches to improve robustness in dairy cows

On a genetic basis, robustness in dairy cows refers to the adaptation ability to a wide range of environmental conditions, i.e, implying selection of genotypes with quite stable genetic values in different environments. Hence, those animals are robust against the impact of possible genotype by environment (G x E) interactions (Rauw and Gomez-Raja, 2015). The concept of G x E interactions applied to production traits in terms of reduced environmental sensitivity contributed to robustness in dairy cows, without extending recording schemes for novel functional traits. Environmental sensitivity is associated with disease susceptibility, and changes of immune response mechanisms. In order to improve disease resistance via direct breeding strategies, health traits on the basis of producer-recorded data were included gradually into overall dairy cattle breeding goals over a period of ~ 20 years.

The classical approach: Detection and use of genotype by environment interactions for conventional traits

Gause et al. (1947) introduced the term environmental sensitivity or phenotypic plasticity, which describes the rate of phenotypic alterations in response to changes in the environment. As outlined by de Jong and Bijma (2002), robust genotypes express quite constant phenotypes across environments. Oppositely, genotypes with high variable phenotypes are more sensitive to environmental impact. Plasticity in protein turnover, antibody production, immune cell response, or in stress-response neuroendocrine axis function is related to robustness, due to the strong effects on a wide range of physiological processes (e.g., reproduction, metabolism, immune response, combating infections) (Magombedze et al., 2013; Mormède et al., 2011).

Phenotypic plasticity also explains G x E interactions. Differences in trait reactions between genotypes in different environments indicate possible G x E interactions. Methodological concepts for the detection of G x E interactions based on reactions of ‘simple’ production or fertility traits in dependency of an environmental descriptor (e.g., temperature, disease exposure, feeding regime). As a simple approach, multiple trait animal or sire models were applied, implying the definition of discrete environmental classes (e.g., Haile-Mariam et al., 2008; Nauta et al., 2006). In multiple trait models, the trait of interest was defined as a separate

trait in different environments. The proof for possible G x E interactions bases on genetic correlations lower than 0.80 between same traits from different environments (Robertson, 1959).

G x E interactions for production and fertility traits were detected when stratifying data according to geographic differences within countries (e.g., Haile-Mariam et al., 2008; König et al., 2005), but also across country borders (e.g., Montaldo et al., 2017). König et al. (2005) only identified indications for G x E interactions when considering several environmental descriptors simultaneously, e.g., herd location and herd size. Such findings were motivation for a "borderless clustering" approach in international genetic evaluations (Weigel and Rekaya, 2000). Furthermore, genetic correlations were generally smaller for low heritability functional traits compared to production traits. For example, Boettcher et al. (2003) created the discrete production environments "grazing" and "conventional". The genetic correlation for calving interval was 0.64, but close to 1 for milk yield. One explanation for obvious G x E of fertility traits addresses pronounced environmental sensitivity in physiological hormone levels and immune functions, compared to the stable hormonal impact on milk production traits (Chagas et al., 2007). Nauta et al. (2006) reported genetic correlations slightly below or larger than 0.80 between organic and conventional protein yield. On the other hand, for different production systems in Australia, the genetic correlation for low heritability pregnancy rates was only 0.37 (Haile-Mariam et al., 2008). For low genetic correlations across environments, an appropriate breeding objective for a given environment should contain the trait-associated environment with appropriate index weights. This is already current practice in many other species' breeding where selection focusses on field traits, but many data, however, coming from station data (Cardoso and Tempelmann, 2012).

An alternative way to detect and to use G x E is the application of random regression or reaction norm models to longitudinal data, which enables estimations of genetic (co)variance components and estimated breeding values (EBV) for a broad grid of continuous environmental variables (e.g., Calus and Veerkamp, 2003). Utilized continuous environmental descriptors reflected the herd management (e.g., average herd milk production, body condition score (BCS), persistency), or climate characteristics (e.g., temperature-humidity index (THI)) (Calus and Veerkamp, 2003; Fikse et al., 2003; Haile-Mariam et al., 2008). BCS as a continuous herd descriptor reflects the dairy cow energy balance, being strongly related to disease resistance and tolerance (Calus and Veerkamp, 2003; Fikse et al., 2003). For example, a cow with constantly low BCS scores has no defense mechanisms against invading pathogens. In this regard, Calus and Veerkamp (2003) estimated sire breeding values for milk production traits of

Dutch dairy cows in dependency of continuous herd BCS. Moreover, lactation persistency was defined as an environmental descriptor reflecting herd management (Calus and Veerkmap, 2003; Fikse et al., 2003), and refers to the rate of decline in the lactation curve after the cows lactation peak. Positive genetic correlations in the range from 0.15 to 0.84 between persistency and relative peak milk yield were estimated, suggesting a higher relative peak milk yield in high persistency herds (e.g., Fikse et al., 2003). Low persistency was strongly associated with increased susceptibility to stress, explaining improved host defense against infectious diseases in persistent herds (Calus et al., 2013). Twomey et al. (2018a) identified re-rankings of sires for fertility traits across environments differing in endoparasite load, i.e., using herd prevalences for the liver fluke *Fasciola hepatica* to measure genetic variability in resilience. In this regard and from a breeding perspective, resilience is defined as the ability of an animal to maintain performance during any environmental perturbation, including high pathogen loads (Bishop, 2012; Colditz and Hine, 2016). No G x E interaction was detected for milk production traits, reflecting that fertility is more influenced by *F. hepatica* infections compared to production traits (Twomey et al., 2018a). This was also shown in own studies for different Black and White dairy cattle selection lines being infected with *F. hepatica* (May et al., 2018).

Tolerance to heat stress is a major component of robustness, refecting the cow's ability to maintain performance during challenging climatic conditions. Possible G x E interactions were evaluated across levels of THI (e.g., Brügemann et al., 2013). As reviewed by Carabaño et al. (2017), genetic correlations between productivity of Holstein cattle kept in cold climatic conditions and productivity under heat stress ranged from 0.40 to 0.98. However, for the functional traits conception rate and somatic cell score (SCS), a substantial decline of genetic correlations was observed, especially when correlating measurements from THI in great distance (Brügemann et al., 2013). On a genetic basis, variations of additive-genetic (co)variance components by THI might indicate that different genes are "switched on or off" under certain environmental conditions. Reaction norm models are suitable to analyse G x E, because of the modelling of phenotypic variation as a function of an environmental descriptor. In such perspective, environmental sensitivity describes the first derivative (i.e., the slope) of the defined reaction norm function. A steep slope indicates high environmental sensitivity (e.g., Streit et al., 2012). In a random regression approach, breeding values for the regression coefficient depend on the value of the regression variables. Therefore, breeding objectives, but alo index traits, could be defined by simply fixing the desired target regression value. Another advantage of this type of models is their capacity to accommodate and to use all response data along the trajectory of the regression variables.

The next step: Inclusion of producer health data in breeding objectives

Genetic evaluations for health traits are mostly based on producer-recorded data. Most commonly, diagnosis keys or recording guidelines depict hierarchical entry systems for the overall disease categories claw disorders, mastitis, fertility disorders, and metabolic disorders, with possibilities for single disease trait specifications. In most studies, heritabilities for binary defined producer-recorded disease traits were quite low. Zwald et al. (2004) estimated heritabilities for producer-recorded binary health data (presence or absence of the respective disease) in the range from 0.07 and 0.18 for the economic relevant disorders mastitis, lameness, ketosis, and cystic ovaries. Heritabilities are in line with estimates by Neuenschwander et al. (2012), reporting heritabilities from 0.02 to 0.21 for producer health data from Canada. Low disease incidences and poor data quality, because of subjective trait recording, makes genetic evaluations for health traits to a challange (Neuenschwander et al., 2012). Advances for developing a routine genetic evaluation for metabolic disease traits were made in Canada by combining producer health data with disease indicator measurements from the laboratory, e.g., ß-hydroxybutyrate (BHB) (Pryce et al., 2016). Heritabilities for objective laboratory measurements were larger compared to subjective binary producer scores, suggesting additional selection response when including BHB into overall breeding indices.

Recently, Martin et al. (2018) reviewed breeding strategies to improve mastitis resistance. In Scandinavia, resistance to mastitis (based on a veterinarian recording scheme for unspecific CM) was included into overall breeding goals since the 1960s. In Canada, a national genetic evaluation for producer-recorded CM was implemented in 2014. Heritability estimates for producer-recorded CM were quite small, in the range from 0.01 and 0.10 (Carlén et al., 2004; Zwald et al., 2004). Simple recording of CM (presence/absence) does not cover intensities of infection and udder pathogen burden. Thus, the estimation of genetic parameters for major specific pathogens was proposed as a pre-requisite for the development of udder health indices with different economic weights for different pathogens. However, also heritabilites for different major pathogens causing CM were quite small, and the specification of pathogens via bacteriological analyses is currently too expensive for commercial breeding applications (Sorensen et al., 2009).

Resistance to claw disorders considers the simple trait lameness (presence/absence), or individual claw disorders with high incidences (e.g., dermatitis digitalis (DD)). Again, also for producer-recorded claw disorders, heritabilities were small, in a narrow range from 0.03 to 0.08 (Neuenschwander et al., 2012; Zwald et al., 2004). For claw disorders, the application of

detailed scoring systems improved phenotype quality, being a better data basis to infer the genetic background of the respective disease. As elaborated by Schöpke et al. (2015), heritabilities for DD increased when applying a more precise scoring system, considering the variety of disease stages.

Potential of (novel) biomarkers to improve robustness to environmental stressors and disease

Biomarkers are objectively measurable indicators to infer biological processes, physiological conditions (e.g., reaction to environmental stressors) or giving a prognostic or diagnostic information value in the face of infectious or non-infectious diseases (for definition, see Biomarkers Definitions Working Group, 2001). Potential biomarkers include enzymes, serum metabolites, hormone levels, or characteristics of body fluids. New technologies such as mid-infrared spectrometry (MIR) in milk can be used to monitor cow health. MIR is a "by-product" from routine milk analysis and provides suitable biomarkers (e.g., milk fatty acids (MFA)) for monitoring diseases (e.g., mastitis, ketosis) (Bastin et al., 2013; Pryce et al., 2016). Novel biomarkers as actually used in quantitative-genetic studies are listed in Table 1, all offering potential for the identification of genetically robust dairy cattle.

Biomarkers for udder health

Regarding resistance to udder infections, test-day SCS and lactation mean SCS are reliable biomarkers, with heritabilities in a range from 0.05 to 0.19 (Martin et al., 2018). Large positive genetic correlations between SCS and clinical mastitis (CM) were motivation for the inclusion of SCS as an indicator trait into selection indexes, allowing the consideration of mastitis resistance into breeding goals (Martin et al., 2018). Further alternative SCS trait definitions, e.g., SCS only from early lactation, or SD of SCS, explained a larger proportion of breeding value variations for mastitis resistance, compared to lactation SCS (Martin et al., 2010).

Minerals (e.g., Ca, K, Mg, Zn, Se, P) or mineral content measured via MIR in bovine milk can be used as potential biomarkers to improve mastitis resistance (Egger-Danner et al., 2015). As shown in Table 1, heritabilites for minerals in milk ranged from 0.20 for selenium to 0.62 for phosphorus. Moreover, milk glycoprotein lactoferrin (LTF) was proposed as a reliable biomarker for mastitis. LTF as an antibacterial and antifungal molecule plays a crucial role in host defense mechanisms, and reacted better for some type of mastitis pathogens compared to SCS (Farnaud and Evans, 2003). Regarding studies from the past 20 yrs, heritabilities for LTF measured via MIR ranged from 0.20 to 0.22 (Table 1). Lactic acid was suggested as a potential

biomarker for clinical mastitits in early lactation, but heritability estimates were close to zero in Danish Holstein cows (Table 1).

During the past years, electrical conductivity (EC) in milk was considered as a novel biomarker in selection indices for improved udder health. Heritabilities for test-day EC records ranged between 0.22 and 0.39 (Table 1). Large genetic correlations (0.75) between test-day EC records and mastitis indicate genetic improvements for mastitis resistance through selection on reduced EC (Norberg et al., 2006). For EC generated from automatic milking systems, larger heritability estimates between 0.37 and 0.51 were reported (Table 1). Generally, automatic milking systems are a new technical opportunity to generate a longitudinal data structure for objectively recorded health indicator traits (Santos et al., 2018).

Biomarkers for metabolic health disorders

Biomarkers to measure energy balances and metabolic diseases include fat-protein-ratio (FPR), ketone bodies (e.g., BHB, acetone), non-esterified free fatty acids (NEFA) or phospholipids, glucose and insulin growth factor 1 (IGF-1), which can be measured in blood, urine and in milk. Currently, the FPR generated from routine milk recording data, is a selection criterion to improve metabolic stability (Koeck et al., 2014). A FPR above 1.5 indicates abnormally high lipolysis, resulting in energy deficiency and metabolic stress in early lactation. Positive genetic correlations from 0.30 to 0.63 between FPR and metabolic diseases (e.g., ketosis, acidosis) were reported (Pryce et al., 2016), suggesting utilization of FPR as a “low cost indicator” for the metabolic disease status. However, FPR is highly affected by feeding regime and BCS. Hence, from a genetic perspective, BHB might be a better biomarker to predict ketosis. Ketosis is associated with an increase of the metabolite BHB in blood (gold standard for diagnosis) or in milk. Heritabilities for BHB were in a range from 0.04 to 0.40 (Table 1), depending on lactation stage. Thus, BHB was used as a biomarker in genetic or genomic approaches to breed cows with decreased susceptibility to ketosis (Pryce et al., 2016). Genetic correlations between BHB with FPR ranged from 0.12 to 0.49, and between BHB with clinical ketosis from 0.37 to 0.75 (Koeck et al., 2014; Pryce et al., 2016). Heritabilities for acetone concentration ranged from 0.01 to 0.29 in early lactation (Table 1). Moreover, NEFA in plasma as a product of body fat mobilization might be suitable indicators for the overall metabolic health status. Heritabilities for NEFA from RRM were in the range from 0.08 to 0.35. NEFA peaked in week one of lactation and provides a substrate for BHB, resulting in the highest levels of BHB in the second week of lactation (Klein et al., 2018). In this regard, a higher accuracy of predicting ketosis can

be achieved when considering more than one biomarker (BHB together with NEFA or FPR) from MIR (Grelet et al., 2016).

The consideration of MFA as biomarkers to detect hyperketonemia and periods of negative energy balance is of growing interest. Van Haelst et al. (2008) observed a significant higher secretion of long-chain fatty acid C18:1 *cis*-9 in milk in subclinical ketosis diagnosed cows. Moreover, an increase in C18:1 *cis*-9 was identified as a biomarker for a decrase in energy balance due to reduced feed intake and nutrient absorption, especially under hot conditions (Hammami et al., 2015). Bastin et al. (2013) estimated heritabilities for a broad range of longitudinal MFA (e.g., butanoic acid (C4:0), caprylic acid (C8:0)). As shown in Table 1, heritabilities for short- and medium chain MFA ranged from 0.35 to 0.59, depending on the lactation stage. Heritabilities for long-chain fatty acids were generally lower (0.18).

Tetens et al. (2015) showed that phospholipids as fatty acids in milk (e.g., phosphatidylcholine (PC), glycerophosphocholine (GPC) and the ratio of both metabolites) are suitable prognostic biomarker for ketosis. A heritability of 0.48 for the metabolite GPC was estimated in a population of Danish Holstein cows (Table 1). Hence, selection of cows with a high GPC level or a high GPC/PC ratio is associated with improved ketosis resistance in early lactation. Currently, intensive recording of phospholipids is very expensive and logistics are difficult to implement in practice, thus, also hampering breeding efforts (Tetens et al., 2015). Further approaches suggested consideration of parameters from intravenious glucose tolerance test (ivGTT) for breeding aspects (Pieper et al., 2016). Heritabilities in Holstein Friesian (HF) bulls ranged from 0.12 to 0.43 for different blood glucose parameters (e.g., fasting glucose concentration, glucose half-life period) (Pieper et al., 2016).

A negative energy balance during the early lactation period is associated with major alterations in the growth hormone IGF-1. Piechotta et al. (2012) showed that prepartum plasma IGF-1 concentrations were lower in cows with postpartum diseases, revealing the potential of IGF-1 in genetic and genomic approaches. Genetic variation for IGF-1 was detected in dairy cows in Poland (Table 1), with a moderate heritability of 0.35.

Rumen microbiome composition as a biomarker

Recently, biomarkers from the rumen microbiome were suggested to study metabolic performances and disease susceptibilities in dairy cattle (Jewell et al., 2015). There is evidence for the existence of inter-individual differences in microbiome compositions (Lin et al., 1997), hypothesising that the host genome has substantial influence on the rumen microbiome composition. In addition, variations in feed efficiency were associated with differences in

rumen microbial community compositions, microbiome-host interactions, or both (Jewell et al., 2015). Hernandez-Sanabria et al. (2013) showed that variations in particular microbial phylotypes due to host sire effects influenced rumen microbial metabolic processes, and ultimately determined residual feed intake (RFI). Thus, interactions between host genotypes and environmental factors (e.g., diet) regulate presence or absence of particular microbes, as well as RFI. Additionally, interactions between an animal's genome and rumen microbiome composition have impact on the occurence and resistance to a number of infectious and non-infectious diseases. In consequence, cows with a specific ruminal composition might be more resistant against specific infectious and non-infectious diseases. Variability and specificity of MFA was associated with the cow energy status and rumen health, indicating cow robustness (Bastin et al., 2013). Heritabilities for MFA ranged between 0.03 and 0.59 (Table 1). However, because of the novelty of this approach, genetic parameters for direct rumen health or microbial composition traits are lacking. Only Sasson et al. (2017) analysed relationships between host genetic architectures with phylogenetic and functional compositions of the rumen microbiome. They identified several microbial groups being heritable and linked to rumen metabolic parameters and productivity in dairy cows. Hence, there is evidence that host genetic variation is associated with specific microbes, and that the bovine rumen microbiome includes heritable components, encouraging further studies on a molecular genetics scale. So far, molecular host response mechanisms determining variation in the microbial populations, are unknown.

Biomarkers for heat stress

Hot temperatures and humidity directly affect physiology (e.g., increase in respiration or heart rate), feed intake, endocrine mechanisms and the effective immune response in dairy cattle, leading to enhanced disease susceptibility (Das et al., 2016). Sanders et al. (2009) observed increased incidence rates for claw disorders with increased air temperature. Increasing THI often causes high reproduction rates for a broad range of pathogens (e.g., bacterials, parasites). Thus, the risk for mastitis and endoparasite infections increases, leading to measurable changes in disease related biomarkers (e.g., SCS). Heat stress response is regulated via endocrine, metabolic, physiological and cellular mechanisms, resulting in up- and downregulations for a number of metabolites (Al-Kanaan et al., 2016; Hammami et al., 2015). Under heat stress, plasma levels of urea and insulin increases, while plasma glucose decreases (Wheelock et al., 2010). Alterations in these biomarkers are directly linked to cows energy balance and might increase susceptibility to metabolic disorders (e.g., ketosis) (West 2003). In consequnece, biomarkers responding in the course of a metabolic disease (e.g., creatine, acetoacetate,

arachidonic acid, BHB), are very sensitive in heat stressed lactating dairy cows (Carabaño et al., 2017). Hammami et al. (2015) measured fatty acids in milk via MIR, and identified C18:1 *cis*-9 as the most sensitive biomarker for heat stress in HF cows. A possible explanation might be that composition of rumen microbiome was significant different in various temperatures (20°C vs. 28°C vs. 33°C) (Tajima et al., 2006), implying a change of nutrient composition in the rumen and fatty acids in milk. In addition, milk protein fractions such as caseins were utilized as suitable biomarkers for heat stress (Carabaño et al., 2017). In recent years, heat shock proteins (HSP) as an expression of cellular stress during heat stress impact have been carefully evaluated. Heat shock protein 70 (HSP70) concentration in plasma and plasma HSP72 were suggested as biomarkers for chronic heat stress, environmental stress and for disease resistance in HF dairy cattle (Carabaño et al., 2017).

Al-Kanaan (2016) analysed different bull semen characteristics and body fluids on a quantitative-genetic scale in dependency of THI. They identified a strong detrimental temperature x humidity - effect on bull semen parameters, and alterations of bull semen breeding values, beyond THI 60. Thus, selection of sires according to THI specific breeding values for specific semen traits and body fluids might be a further strategy to identify sires with improved resistance to hot climatic conditions. Further physiological traits as indicators for resilience to heat stress include measurements of body temperature (rectal, vaginal, skin), pulse and respiration rate (Table 1). Reported heritabilities for body temperature traits ranged from 0.02 to 0.17, while heritabilities for pulse and respiration rate were in a range from 0.05 to 0.07 (Table 1).

Biomarker for endoparasite infections

In pasture-based production systems, endoparasite infections caused by gastrointestinal nematodes (GIN) and liver flukes (*Fasciola hepatica*) are of increasing economic importance, because of strong associations with impaired dairy cow health. Biomarkers for host resistance (i.e., ability to control pathogen burden) or tolerance (i.e., ability to limit the impact of a given pathogen burden on performance) to parasitic infections include biochemical (e.g., serum pepsinogen or albumin level), immunological (serum or milk antibodies) and parasitological (e.g., faecal egg count, worm parameters, helminth-specific antigen, DNA) markers. Serum or milk antibodies (different isotypes of immunoglobulines) and FEC are the most common used indicators to measure resistance. For GIN infections in dairy cattle, heritabilities ranged from 0.05 to 0.21 for FEC, but the heritability for antibody levels measured via ELISA was only 0.07 (Table 1). In literature, negative genetic correlations were reported between FEC and antibody

levels (IgG1, IgG2, IgA, IgM) for GIN infections in cattle and small ruminants. Thus, breeding for enhanced resistance to endoparasite infections is possible via selection for lower FEC, or via higher antibody titres. May et al. (2017) estimated negative genetic correlations up to -0.40 between GIN infections detected by FEC with fat percentage and with protein percentage throughout lactation. In their study, genetic correlations between FEC and milk yield were close to zero. Accordingly, genetic correlations for the antibody response to the gastrointestinal nematode *Ostertagia ostertagi* with milk production traits were close to zero (Twomey et al., 2018b).

For liver fluke infections, Twomey et al. (2018b) used the biomarker "IgG antibody titres against *F. hepatica"* in Irish dairy cows, and estimated a heritability of 0.13 (Table 1). A heritability of 0.33 was estimated for the biomarker "FEC of *F. hepatica*" in German Holstein dairy cows (May et al., 2017; see Table 1). Twomey et al. (2018b) estimated negative genetic correlations in a low range (-0.04 to -0.14) between IgG antibody titres against *F. hepatica* and milk production traits. May et al. (2017) found negative correlations up to -0.50 between FEC of *F. hepatica* and protein %. Currently, utilization of FEC or antibody levels to predict resistance or tolerance to endoparasite infections in dairy cows is not feasible, due to the costs and difficulties of measuring phenotypes in a commercial production environment. However, the application of suitable traits measuring overall immune response to pathogenic agents will play a greater role in future selection strategies, instead a selection focus on specific infectious diseases.

Table 1. Heritability estimates in HF dairy cows for novel biomarkers (somatic cell count and fat-to-protein ratio not included) as indicators of resistance, tolerance and resilience associated traits in studies as from 2000 (ordered chronologically for each biomarker).

Biomarker	No. of cows	Heritability	Comment	Reference
Udder health				
Mineral content	1,860	0.20 to 0.62	L1	van Hulzen et al. (2009)
	44,000	0.34 to 0.55	L1, MIR	Soyeurt et al. (2012)
Lactoferrin (LTF)	1,773	0.20	All lactations, MIR	Soyeurt et al. (2007a)
	6,256	0.22	All lactations, MIR	Arnould et al. (2009)
Lactic acid	371	0.00	L1-L3	Buitenhuis et al. (2013)
Electrical conductivity (EC)	3,500	0.27 to 0.39	L1	Goodling et al. (2000)
		0.21 to 0.23	L2	
	2,102	0.28	L1	Norberg et al. (2004)
		0.26 to 0.36	L1, RRM	
	421	0.51	All lactations, AMS	Juozaitiené et al. (2015)
	922	0.37 to 0.46	L1-5, AMS	Santos et al. (2018)
Metabolic disease/rumen microbiome				
ß-hydroxybutyrate (BHB)	175	0.25	Heifers before calving	Oikonomou et al. (2008)
		0.08 to 0.40	L1, RRM	
	1,615	0.17	All lactations, plasma BHB	Van der Drift et al. (2012)
	1,565	0.16	All lactations, milk BHB	
	61,331	0.12	L1, first test day (5-40 DIM)	Koeck et al. (2014)
	3,732	0.13	L1	Jamrozik et al. (2016)
	35,575	0.08	>L1	
	7,895	0.04 to 0.17	L1-L3, random regression model	Lee et al. (2016)
	14,397	0.06 to 0.15	L1, different lactation stages	Lee et al. (2017)

Biomarker	No. of cows	Heritability	Comment	Reference
	826	0.25 to 0.37	All lactations, MIR	Belay et al. (2017)
	~ 18,000	0.09 to 0.14	L1-4 milk	Ranaraja et al. (2018)
Acetone	10,375	0.01	All lactations	Wood et al. (2004)
	7,895	0.29	L1	Lee et al. (2016)
	7,895	0.29	L2	
	7,895	0.22	L3	
	1,565	0.10	Milk acetone, all lactations	Van der Drift et al. (2012)
	14,397	0.06 to 0.15	L1, different lactation stages	Lee et al. (2017)
	~ 18,000	0.23 to 0.31	L1-4	Ranaraja et al. (2018)
Non-esterified free fatty acids (NEFA)	142	0.29	Heifers before calving	Oikonomou et al. (2008)
	6,015	0.08 to 0.35	L1, RRM	
Milk fatty acids (MFA)	26,166	0.18 to 0.44	L1, MIR	Bastin et al. (2011)
	7,700	0.05 to 0.38	All lactations, MIR	Soyeurt et al. (2007b)
	11,626	0.14 to 0.42	L1, RRM, MIR	Soyeurt et al. (2008)
	1,918	0.09 to 0.54	L1	Stoop et al. (2008)
	990	0.03 to 0.19	L1-7	Mele et al. (2009)
	44,000	0.26 to 0.59	L1, MIR	Soyeurt et al. (2012)
	37,768	0.22 to 0.46	L1, MIR	Bastin et al. (2013)
	22,566	0.21 to 0.42	L2	
	8,221	0.18 to 0.39	L3	
Phospholipids (glycerophosphocholine)	371	0.48	L1-3	Buitenhuis et al. (2013)
Glucose	192	0.21	L1-3	Ahn et al. (2006)
	174	0.37	Heifers before calving	Oikonomou et al. (2008)
	6,015	0.12 to 0.39	L1	
Insulin growth factor 1 (IGF-1)	75	0.35	L1	Grochowska et al. (2001)

Biomarker	No. of cows	Heritability	Comment	Reference
Heat stress				
Rectal temperature	1,695	0.17	All lactations	Dikmen et al. (2012)
	238	0.07	All lactations	Al-Kanaan et al. (2016)
Vaginal temperature	238	0.04	All lactations	Al-Kanaan et al. (2016)
Skin temperature	238	0.02 to 0.04	All lactations	Al-Kanaan et al. (2016)
Respiration rate	238	0.05	All lactations	Al-Kanaan et al. (2016)
Pulse rate	238	0.07	All lactations	Al-Kanaan et al. (2016)
Endoparasite infections				
Gastrointestinal nematodes				
Faecal egg counts (FEC)	1,419	0.21	L2-4	Coppieters et al. (2009)
	4,053	0.07	L1-4	
	1,166	0.05 to 0.06	All lactations	May et al. (2017)
Antibody levels	10,879	0.07	All lactations	Twomey et al. (2018)
Liver flukes (*F. hepatica*)				
Faecal egg counts (FEC)	1,166	0.33	All lactations	May et al. (2017)
Antibody level	10,879	0.13	All lactations	Twomey et al. (2018)

AMS = automatic milking system; DIM = Days in milk; HF = Holstein Friesian; L = lactation; RRM = random regression model; MIR = assessed by mid-infrared spectrometry

Inclusion of immunity traits into breeding objectives

The immune system includes two components: The innate (non-specific) and the adaptive (specific) immune system. During the last years, breed-dependent differences and suitable IR traits were investigated for both host defense mechanisms (i.e, the innate and the adaptive immune system), suggesting the potential of IR traits as useful selection markers, in order to achieve enhanced disease resistance. Immune response mechanisms are primarily linked to resistance against infectious diseases. Nevertheless, also the interplay between the immune status and non-infectious diseases (e.g., metabolic health) was investigated in dairy cattle (e.g., Thompson-Crispi et al., 2013). An increase in incidences of various diseases (e.g., mastitis, ketosis) around calving and during the postpartum period was explained trough alterations in response of important IR mediators.

Immune response traits

The genetic background of IR traits has been studied during the past 30 years in dairy cows. In Norwegian Red dairy cattle, heritabilities for serum proteins (complement, conglutinin, lysozyme) and immunoglobulins (Ig) were larger than 0.50 (Lie, 1979). Detilleux et al. (1994) investigated the genetic variation of serum Ig concentrations and neutrophil functions in HF cows for different immunosuppression stages (day 35 prepartum to day 35 postpartum). Moderate to large heritabilities up to 0.84 were estimated for different Ig isotypes (IgG1, IgG2, IgM), depending on the immunosuppression stage. The lactation stage had significant impact on Ig levels, and heritabilities were larger in the periparturient period (Detilleux et al., 1994; Mazengera et al., 1985; Wagter et al., 2000). In dairy cattle, the neutrophil function plays a decisive role as the first defense against udder infections. Heritabilities for specified IR traits being related to mastitis (e.g., neutrophil functions) differed from 0.20 to 0.70 in periparturient Holstein cows (Detilleux et al., 1994).

Abdel-Azim et al. (2005) used a combination of immunologically controlled infectious diseases induced by pathogens (e.g., uterine infections, respiratory diseases) to introduce the overall immune trait ‘generalized immunity (GI)’ in US HF cows. The heritability in this study for GI was 0.20. Further advances have been made in exploring humoral or antibody-mediated IR (AMIR), and cellular-mediated IR (CMIR) of the bovine immune system. Immunoglobulins (antibodies) are the mediators of AMIR, produced from B cells as a reaction in the presence of extracellular pathogens. Antigen-presenting cells and cytotoxic T cells stimulate CMIR, being responsible for the protection against intracellular pathogens. A Canadian research group demonstrated selective breeding possibilities for high responders (HR), average responders

(AR) or low responders (LR), based on AMIR and on CMIR (Guelph's patented High Immune Response technology, patent number: CA2255423A1). In this regard, a standardized immunization or IR-testing protocol is used to capture CMIR and AMIR (e.g., Thompson-Crispi et al., 2012 and 2013). In this protocol, hen-egg white lysosyme (HEWL) or ovalbumin are the antigens to measure induced antibody (mostly IgG1 and IgG2) response for AMIR at different days after immunization. Delayed-type hypersensitivity to the yeast fungi *Candida albicans*, or to the intracellular bacterium *Mycobacterium avium* subsp. *paratuberculosis*, induced CMIR.

Heritabilities for CMIR ranged from 0.19 to 0.54 (Heriazon et al., 2013; Thompson-Crispi et al., 2012). Heritabilities for AMIR were in a broader range from 0.13 to 0.88, depending on the antigen isotype (Heriazon et al., 2013; Thompson-Crispi et al., 2012; Wagter et al., 2000). In most cases, genetic correlations between CMIR and AMIR were negative (e.g., Heriazon et al., 2013; Hernandez 2006; Thompson-Crispi et al., 2012), indicating that selection of cows with increased resistance to extracellular pathogens (e.g., helminths) might be associated with increasing susceptibility to intracellular pathogens (e.g., bacteria, viruses, protozoa), and vice versa. For example, Twomey et al. (2018b) identified antagonistic genetic relationships between antibody response to the protozoan parasite *Neospora caninum* with the antibody response to the helminths *F. hepatica* (-0.29) and *O. ostertagi* (-0.67) in Irish dairy cattle.

Relationships between immune response traits with production and reproduction traits

A more balanced and robust immune system may be associated with decreased productivity, because a cow allocates more resources (e.g., nutrients) to the immune sytem. However, Thompson-Crispi et al. (2012) showed that selection on high IR improved milk yield in dairy cattle. Herizazon et al. (2013) reported only low phenotypic correlations from 0.01 to 0.18 between CMIR or AMIR with milk production traits. On a genetic scale, the correlation was 0.05 between AMIR and protein percentage, and 0.18 between AMIR and fat percentage (Heriazon et al., 2013). Interestingly, the same study identified negative genetic correlations between CMIR and fat or protein percentage (-0.11 to -0.15). Samoré et al. (2010) estimated positive genetic correlations between fat percentage and functional longevity. Hence, contiuous breeding on effective and high AMIR reflecting best adaptation to a wide range of extracellular pathogens, positively influences longevity. Thompson-Crispi et al. (2012) reported a positive genetic correlation of 0.16 between CMIR and milk yield in first parity cows. Mazengera et al. (1985) estimated positive genetic correlations in the range from 0.13 to 0.63 between specific IR traits (IgG2 and IgA) with 305-d milk and fat percentage, but genetic correlations between

IgM with 305-d milk and fat percentage were negative (-0.83 and -0.34, respectively). A negative genetic correlation of -0.18 between IgG isotype and daily milk yield was estimated (Table 2). A complete overview including genetic correlations between IR traits and production and fertility traits is given in Table 2.

Improved IR had beneficial effects on reproductive traits (Thompson-Crispi et al., 2012). Phenotypically, correlations between IR traits AMIR and CMIR with days to first service were lower than -0.11 (Heriazon et al., 2013). Low, but positive genetic correlations in the range from 0.16 to 0.20 between non-return rates and IR traits (AMIR, CMIR) were estimated in Canadian heifers (Thompson-Crispi et al., 2012). The genetic correlation between gestation length and AMIR was favorable (-0.17) (Thompson-Crispi et al., 2012)

Relationships between immune response traits and health traits

Sires with high IR improved colostrum quality and enhanced resistance to non-infectious and infectious diseases in their daughters (e.g., Wagter et al., 2000). Abdel-Azim et al. (2005) estimated correlations among predicted sire transmitting abilities for clinical disease traits and IR traits. Correlations between GI (simply defined as a combination of infectious diseases) and non-infectious diseases were lower (0.01 to 0.20) compared with correlations between GI and infectious diseases (0.56 to 0.79).

A low genetic correlation (0.03) between IgG and blood glucose was reported in Korean Holstein cows (Table 2). Blood glucose is a biomarker for non-infectious diseases or metabolic health. In contrast, the genetic correlation between IgG and SCS (biomarker for mastitis) induced by pathogens was 0.68 (Table 2). Such results indicate that resistance to infectious diseases is primarily regulated by IR genotypes, while resistance to non-infectious diseases strongly depends on environmental factors. Thompson-Crispi et al. (2013) compared the CM incidence rates between HR-, AR- and LR-cows for AMIR and CMIR. In this study, a significant higher incidence rate for CM induced by *Staphylococcus aureus*, E. coli, *Streptococcus spp*. and further bacterial pathogens was identified in LR-AMIR cows compared to HR-AMIR cows. Kelm et al. (1997) estimated negative correlations between EBV for specific IR traits (e.g., Ig isotypes, neutrophil function) with EBV for SCS. Negative correlations indicate that cows with low EBV for SCS tend to have neutrophils with a greater functional ability at maximal immunosuppression. Moreover, regarding neutrophil functions, significant progeny group differences were detected (Kelm et al., 1997). Bannerman et al. (2008) identified breed-dependent differences for IR traits (e.g., neutrophil cell count, T lymphocytes), which are involved in host immune mechanisms against mastitis, induced by S.

aureus. In this study, HF cows showed stronger neutrophil response to udder infections than Jersey cows. Banos et al. (2013) identified significant variations for cellular immune traits (e.g., percentage of neutrophils or T lymphocytes in blood) in different genetic lines of Scottish HF dairy cows. Next to breed specific variations in immune response, higher variations in immunoglobulin combinatorial diversity were detected in Aubrac compared with HF or German Black Pied cattle (Walther et al., 2016). Immunoglobulin diversity reflects the cow's adaptation to antigens under certain environmental conditions. Thus, phenotypic differences for same genotypes in different environments regarding antibody diversity can be expected.

Conclusions

From a conventional and novel breeding perspective, improving robustness implies i) to select animals with high and stable breeding values for production or fertility traits in the course of environmental alterations (concept of G x E interactions), ii) to include producer-recorded health data in future breeding objectives, iii) to focus on objectively measurable indicator traits from routine milk recording schemes and / or on novel biomarkers. Generally, as outlined in the present review, more accurate and objective phenotyping strategies allow more accurate genetic parameter estimations, and larger heritabilties. In such perspective, it is imperative to evaluate a broad pattern of biomarkers being linked to environmental response and disease resistance. A quite large number of biomarkers being linked to environmental sensitivity and disease resistance have been detected (e.g., C18:1 *cis*-9 milk fatty acid, BHB). Rapid progress in breeding advances for overall robustness (i.e., those animals being best adapted to environmental stressors and less susceptible to disease) suggest the inclusion of biomarkers being sensitive for more than one trait into selection indices. In dairy cattle, an obvious interaction between adaptation to changes in environment, composition of rumen microbiome, metabolism and immune response mechanisms exist, affecting individual's resistance to disease. In consequence, new approaches aiming on enhanced disease resistance focus on IR breeding strategies. Generally, genetic correlations between IR traits and performance and health traits were low to moderate, but breed specific differences in immune response reflect variability for IR traits under identical environmental conditions. Nevertheless, the optimal breeding strategy remains unclear, i.e., utilization of adaptive IR, or using specific disease traits or appropriate biomarkers. Hence, it is imperative in ongoing studies to develop correct weightings and appropriate methods to accommodate all phenotypic sources simultaneously via selection index methodology.

Table 2. Literature overview for genetic correlations between objectively measurable immune response traits and production traits in dairy cattle.

	Immune response traits						
Trait	AMIR	CMIR	IgA	IgG	IgM	Comment	Reference
Production trait							
Milk yield			0.37	0.36	-0.83	Cows	Mazengera et al. (1985)
				-0.18		Cows	Ahn et al. (2006)
		0.16				Cows	Thompson-Crispi et al. (2012)
	-0.06	0.09				Cows	Heriazon et al. (2013)
Milk fat	0.10	0.00				Cows	Heriazon et al. (2013)
Fat-%	0.18	-0.11				Cows	Heriazon et al. (2013)
			0.63	0.13	-0.34	Cows	Mazengera et al. (1985)
Milk protein	-0.04	0.04				Cows	Heriazon et al. (2013)
Protein-%	0.05	-0.15				Cows	Heriazon et al. (2013)
SCS				0.68		Cows	Ahn et al. (2006)
	0.05	-0.09				Cows	Heriazon et al. (2013)
56-d non-return rate	0.16					Heifers	Thompson-Crispi et al. (2012)
Non-return rate	0.21	-0.22				Heifers	Heriazon et al. (2013)
	0.15	-0.22				Cows	Heriazon et al. (2013)
No. of services	0.20					Heifers	Thompson-Crispi et al. (2012)
Age at first service	0.05	0.00				Cows	Heriazon et al. (2013)
First service to conception	0.18					Heifers	Thompson-Crispi et al. (2012)
Calving to first service	0.03	-0.01				Cows	Heriazon et al. (2013)
Daughter calving ease	-0.19					Heifer	Thompson-Crispi et al. (2012)
Calving ease	-0.23	0.11				Cow	Heriazon et al. (2013)
Maternal calving ease	-0.12	0.13				Cow	Heriazon et al. (2013)
Daughter fertility	0.13	-0.22				Cow	Heriazon et al. (2013)
Gestation length		0.17				Heifer	Thompson-Crispi et al. (2012)
	-0.17					Cows	Thompson-Crispi et al. (2012)

Trait	Immune response traits					Comment	Reference
	AMIR	CMIR	IgA	IgG	IgM		
Functional trait							
Blood glucose				0.03			Ahn et al. (2006)
Somatic cell score				0.68			Ahn et al. (2006)

AMIR = antibody mediated immune response; CMIR = cellular mediated immune response; IgA = Immunolobulin A; IgG; Immunoglobulin G; IgM = Immunoglobulin M; SCS = somatic cell score

References

Abdel-Azim GA, Freeman AE, Kehrli ME, Kelm SC, Burton JL, Kuck AL, Schnell S. 2005. Genetic basis and risk factors for infectious and non-infectious diseases in US Holsteins. I. Estimation of genetic parameters for single diseases and general health. Journal of Dairy Science 88:1199-1207.

Ahn BS, Jeon BS, Kwon EG, Ajmal Khan M, Kim HS, Ju JC, Kim NS. 2006. Estimation of genetic parameters for daily milk yield, somatic cell score, milk urea nitrose, blood glucose and immunoglobulin in Holsteins. Asian-Australian Journal of Animal Science 19:1252-1256.

Al-Kanaan A. 2016. Heat stress response for physiological traits in dairy and dual purpose cattle populations on phenotypic and genetic scales. PhD thesis, Faculty of Organic Agriculture, University of Kassel, Kassel, Germany.

Arnould VMR, Soyeurt H, Gengler N, Colinet FG, Georges MV, Bertozzi C, Portetelle D, Renaville R. 2009. Genetic analysis of lactoferrin content in bovine milk. Journal of Dairy Science 92:2151-2158.

Bannerman DD, Springer HR, Paape MJ, Kauf ACW, Goff JP. 2008. Evaluation of breed-dependent differences in the innate immune responses of Holstein and Jersey cows to *Staphylococcus aureus* intramammary infection. Journal of Dairy Research 75:291-301.

Banos G, Wall E, Coffey MP, Banall A, Gillespie S, Russell GC, McNeilly TN. 2013. Identification of immune traits correlated with dairy cow health, reproduction and productivity. PloS One 8:e65766.

Bastin C, Gengler N, Soyeurt H. 2011. Phenotypic and genetic variability of production traits and milk fatty acid contents across days in milk for Walloon Holstein first-parity cows. Journal of Dairy Science 94:4152-4163.

Bastin C, Soyeurt H, Gengler N. 2013. Genetic parameters of milk production traits and fatty acid contents in milk for Holstein cows in parity 1-3. Journal of Animal Breeding and Genetics 130:118-127.

Belay TK, Svendsen M, Kowalski ZM, Adnoy T. 2017. Genetic parameters of blood ß-hydroxybutyrate predicted from milk infrared spectra and clinical ketosis, and their associations with milk production traits in Norwegian Red cows. Journal of Dairy Science 100:6298-6311.

Biomarkers Definitions Working Group. 2001. Biomarkers and surrogate endpoints: preferred definitions and conceptual framework. Clinical Pharmacology & Therapeutics 69, 89-95.

Bishop SC. 2012. A consideration of resistance and tolerance for ruminant nematode infections. Frontiers in Genetics 3:168.

Boettcher PJ, Fatehi J, Schutz MM. 2003. Genotype x environment interactions in conventional versus pasture-based dairies in Canada. Journal of Dairy Science 86:383-389.

Brügemann K, Gernand E, von Borstel UU, König S. 2013. Application of random regression models to infer the gentic background and phenotypic trajectory of binary conception rate by alterations of temperature x humidity indices. Livestock Science 15:389-396.

Buitenhuis AJ, Sundekilde UK, Poulsen NA, Bertram HC, Larsen LB, Sorensen P. 2013. Estimation of genetic parameters and detection of quantitative trait loci for metabolites in Danish Holstein milk. Journal of Dairy Science 96:3285-3295.

Calus MPL, Veerkamp RF. 2003. Estimation of environmental sensitivity of genetic merit for milk production traits using random regression model. Journal of Dairy Science 86:3756-3764.

Calus MPL, Berry DP, Banos G, de Haas Y, Veerkamp RF. 2013. Genomic selection: the option for new robustness traits? Advances in Animal Biosciences 4:618-625.

Carabaño MJ, Ramón M, Díaz C, Molina A, Pérez-Guzmán MD, Serradilla JM. 2017. Breeding and genetics symposium: Breeding for resilience to heat stress effects in dairy ruminants. A comprehensive review. Journal of Animal Science 95:1813-1826.

Cardoso FF, Tempelmann RJ. 2012. Linear reaction norm models for genetic merit prediction of Angus cattle under genotype by environment interaction. Journal of Animal Science 90:2130-2141.

Carlén E, Strandberg E, Roth A. 2004. Genetic parameters for clinical mastitis, somatic cell score, and production in the first three lactations of Swedish Holstein cows. Journal of Dairy Science 87:3062-3070.

Chagas LM, Bass JJ, Blache D, Burke CR, Kay JK, Lindsay DR, Lucy MC, Martin GB Meier S, Rhodes FM, Roche JR, Thatcher WW, Webb R. 2007. Invited review: New perspectives on the roles of nutrition and metabolic priorities in the subfertility. Journal of Dairy Science 90:4022-4032.

Colditz IG, Hine BC. 2016. Resilience in farm animals: biology, management, breeding and implications for animal welfare. Animal Production Science 56:1961-1983.

Coppieters W, Mes THM, Druet T, Farnir F, Tamma N, Schrooten C, Cornelissen AWCA, Georges M, Ploeger HW. 2009. Mapping QTL influencing gastrointestinal nematode burden in Dutch Holstein-Friesian dairy cattle. BMC Genomics 10:96.

Das R, Sailo L, Verma N, Bharti P, Saikia J, Imtiwati, Kumar R. 2016. Impact of heat stress on health and performance of dairy animals: A review. Veterinary World 9:260-268.

De Jong G, Bijma P. 2002. Selection and phenotypic plasticity in evolutionary biology and animal breeding. Livestock Production Science 78:195-214.

Detilleux JC, Koehler KJ, Freeman AE, Kehrli ME, Jr. Kelley DH. 1994. Immunological parameters of periparturient Holstein cattle: genetic variation. Journal of Dairy Science 77:2640-2650.

Dikmen S, Cole JB, Null DJ, Hansen PJ. 2012. Heritability of rectal temperature and genetic correlations with production and reproduction traits in dairy cattle. Journal of Dairy Science 95:3401-3405.

Egger-Danner C, Cole JB, Pryce JE, Gengler N, Heringstad B, Bradley A, Stock KF. 2015. Invited review: Overview of new traits and phenotyping strategies in dairy cattle with a focus on functional traits. Animal 9:191-207.

Farnaud S, Evans RW. 2003. Lactoferrin – a multifunctional protein with antimicrobial properties. Molecular Immunology 40:395-405.

Fikse WF, Rekaya R, Weigel KA. 2003. Assessment of environmental descriptors for studying genotype by environment interaction. Livestock Production Science 82:223-231.

Goodling RC, RogersGW, Cooper JB, Rune B. 2000. Heritability estimates for electrical conductivity of milk and correlations with predicted transmitting abilities for somatic cell scores. Journal of Dairy Science 83(Suppl. 1):71.

Gause GF. 1947. Problems of evolution. Transactions of the Connecticut Academy of Arts and Sciences 37:17-68.

Grelet C, Bastin C, Gelé M, Davière JB, Johan M, Werner A, Reding R, Fernandez Pierna JA, Colinet FG, Dardenne P, Gengler N, Soyeurt H, Dehareng F. 2016. Development of Fourier transform mid-infrared calibrations to predict acetone, ß-hydroxybutyrate and citrate contents in bovine milk through a European dairy network. Journal of Dairy Science 99:4816-4825.

Grochowska R, Sorensen P, Zwierzchowski L, Snochowski M, Lovendahl P. 2001. Genetic variation in stimulated GH release and in IGF-1 of young dairy cattle and their associations with the leucine/valine polymorphism in the GH gene. Journal of Animal Science 79:450-476.

Haile-Mariam D, Carrick MJ, Goddard ME. 2008. Genotype by environment interaction for fertility, survival, and milk production traits in Australian dairy cattle. Journal of Dairy Science 91:4840-4853.

Hammami H, Vandenplas J, Vanrobays ML, Rekik B, Bastin C, Gengler N. 2015. Genetic analysis of heat stress effects on yield traits, udder health, and fatty acids of Walloon Holstein cows. Journal of Dairy Science 98:4956-4968.

Heriazon A, Quinton M, Miglior F, Leslie KE, Sears W, Mallard BA. 2013. Phenotypic and genetic parameters of antibody and dalyed-type hypersensitivity responses of lactating Holstein cows. Veterinary Immunology and Immunopathology 154:83-92.

Hernandez-Sanabria E, Goonewardene LA, Wang Z, Zhou Mi, Moore SS, Guan LL. 2013. Influence of sire breed on the interplay among rumen microbial populations inhabiting the rumen liquid of the progeny in beef cattle. PloS ONE 8:e58461.

Jamrozik J, Koeck A, Kistemaker GJ, Miglior F. 2016. Multiple-trait estimates of genetic parameters for metabolic disease traits, fertility disorders, and their predictors in Canadian Holsteins. Journal of Dairy Science 99:1990-1998.

Jewell KA, McCormick CA, Odt CL, Weimer PJ, Suen G. 2015. Ruminal bacterial community composition in dairy cows is dynamic over the course of two lactations and correlates with feed efficiency. Applied and Environmental Microbiology 81:4697-4710.

Juozaitiené V, Juozaitis A, Brazauskas A, Žymantienė J, Žilaitis V, Antanaitis R, Stankevičius R, Bobinienè R. 2015. Investigation of electrical conductivity of milk in robotic milking system and its relationship with somatic cell count and other quality traits. Journal of Measurements in Engineering 3:63-70.

Kelm Sc, Dettilleux JC, Freeman AE, Kehrli ME, Dietz AB, Fox LK, Butler JE, Kasckovics I, Kelley DH. 1997. Genetic association between parameters of innate immunity and measures of mastitis in periparturient Holstein cattle. Journal of Dairy Science 80:1767-1775.

Koeck A, Jamrozik J, Schenkel FS, Moore RK, Lefebvre DM, Kelton DF, Miglior F. 2014. Genetic analysis of milk ß-hydroxybutyrate and its association with fat-to-protein ratio, body condition score, clinical ketosis, and displaced abomasums in early first lactation of Canadian Holsteins. Journal of Dairy Science 97:7286-7292.

König S, Dietl G, Raeder I, Swalve HH. 2005. Genetic relationships between dairy performance under large-scale farm and family farm conditions. Journal of Dairy Science 88:4087-4096.

Klein S, May K, Scheper C, König S. 2018. Phänotypische und genomweite Assoziationen für ß-Hydroxybutyrat und Aceton in der Milch als potentielle Indikatoren für Ketose in der Frühlaktation. National Animal Breeding Meeting of the German Society of Animal Science 12th and 13th September, Bonn, Germany, p. 37-38.

Lee SH, Cho KH, Park MN, Choi TJ, Kim SD, Do CH. 2016. Genetic parameters of milk ß-hydroxybutyric acid and acetone and their genetic association with milk production traits of Holstein cattle. Asian-Australasian Journal of Animal Sciences 29:1530-1540.

Lee SH, Choi S, Chang-Gwon D, Mahboob A, Do CH. 2017. Genetic parameters of milk ß-hydroxybutyrate acid, milk acetone, milk yield, and energy-corrected milk for Holstein dairy cattle in Korea. Journal of the Korean Data & Information Science Society 28:1349-1360.

Lie O. 1979. Genetic analysis of some immunological traits in young bulls. Acta Veterinaria Scandinavica 20:372-386.

Lin C, Raskin L, Stahl DA. 1997. Microbial community structure in gastrointestinal tracts of domestic animals: comparative analyses using rRNA targeted oligonucleotide probes. FEMS Microbiology Ecology 22:281-294.

Magombedze G, Reddy PBJ, Eda S, Ganusov VV. 2013. Cellular and population plasticity of helper CD4(+) T cell responses. Frontiers in Physiology 4:206.

Mallard BA, Emam M, Paibomesai M, Thompson-Crispi K, Wagter-Lesperance L. 2015. Genetic selection of cattle for improved immunity and health. Japanese Journal of Veterinary Research 63:37-44.

Martin P, Barkema HW, Brito LF, Narayana SG, Miglior F. 2018. *Symposium review*: Novel strategies to genetically improve mastitis resistance in dairy cattle. Journal of Dairy Science 101:2724-2736.

Mazengera KE, Kennedy BW, Burnside EB, Wilkie BN, Burton JH. 1985. Genetic parameters of bovine serum immunoglobulins. Journal of Dairy Science 68:2309-2314.

May K, Brügemann K, Yin T, Scheper C, Strube C, König S. 2017. Genetic line comparisons and genetic parameters for endoparasite infections and test-day milk production traits. Journal of Dairy Science 100:7330-7344.

May K, Brügemann K, König S, Strube C. 2018. The impact of patent *Fasciola hepatica* infections on individual milk production and fertility parameters in dairy cows. 28th Annual Meeting of the German Society for Parasitology, Berlin, Germany, pp.176-177.

Mele M, Dal Zotto R, Cassandro M, Conte G, Serra A, Buccioni A, Bittante G, Secchiari P. 2009. Genetic parameters for conjugated linoleic acid, selected milk fatty acids, and milk fatty acid unsaturation of Italian Holstein-Friesian cows. Journal of Dairy Science 92:392-400.

Montaldo HH, Pelcastre-Cruz A, Castillo-Juárez H, Ruiz-López FJ, Miglior F. 2017. Genotype x environment interaction for fertility and milk yield traits in Canadian, Mexican and US Holstein cattle. Spanish Journal of Agricultural Research 15:e0402.

Morméde P, Foury A, Terenina E, Knap PW. 2011. Breeding for robustness: the role of cortisol. Animal 5:651-657.

Nauta WJ, Veerkamp RF, Brascamp EW, Bovenhuis H. 2006. Genotype by environment interaction for milk production traits between organic and conventional dairy cattle production in The Netherlands. Journal of Dairy Science 89:2729-2737.

Neuenschwander TF-O, Miglior F, Jamrozik J, Berke O, Kelton DF, Schaeffer LR. 2012. Genetic parameters for producer-recorded health data in Canadian Holstein cattle. Animal 6:571-578.

Norberg E, Rogers GW, Goodling RC, Cooper JB, Madsen P. 2004. Genetic parameters for test-day electrical conductivity of milk for first-lactation cows from random regression models. Journal of Dairy Science 87:1917-1924.

Norberg E, Rogers GW, Ødegård J, Cooper JB, Madsen P. 2006. Short communication: Genetic correlation between test-day electrical conductivity of milk and mastitis. Journal of Dairy Science 89:779-781.

Oikonomou G, Valergakis GE, Arsenos, Roubies N, Banos G. 2008. Genetic profile of body energy and blood metabolic traits across lactation in primiparous Holstein cows. Journal of Dairy Science 91:2814-2822.

Piechotta M, Sander AK, Kastelic JP, Wilde R, Heppelmann M, Rudolph B, Schuberth HJ, Bollwein H, Kaske M. 2012. Short communication: Prepartum plasma insulin-like growth factor-I concentrations based on day of insemination are lower in cows developing postpartum diseases. Journal of Dairy Science 95:1367-1370.

Pieper L, Staufenbiel R, Christ J, Panicke L, Müller U, Brockmann GA. 2016. Heritability of metabolic response to the intravenous glucose tolerance test in German Holstein Friesian bulls. Journal of Dairy Science 99:7240-7246.

Pryce JE, Gaddis KP, Koeck A, Bastin C, Adessayed M, Gengler N, Miglior F, Heringstad B, Egger-Danner C, Stock K. 2016. Invited review: Opportunities for genetic improvement of metabolic diseases. Journal of Dairy Science 99:6855-6873.

Ranaraja U, Cho KH, Park MN, Kim SD, Lee SH, Do CH. 2018. Genetic parameter erstimation for milk ß-hydroxybutyrate and acetone in early lactation and its association with fat to protein ratio and energy balance in Korean Holstein cattle. Asian-Australasian Journal of Animal Science 31:798-803.

Rauw WM, Gomez-Raja L. 2015. Genotype by environment interaction and breeding for robustness in livestock. Frontiers in Genetics 6:310.

Robertson A. 1959. The sampling variance of the genetic correlation coefficient. Biometrics 15:469-485.

Samoré AB, Rizzi R, Rossoni A, Banato A. 2010. Genetic parameters for functional longevity, type traits, somatic cell scores, milk flow and production in the Italian Brown Swiss. Italian Journal of Animal Science 9:e28.

Sanders AH, Shearer JK, De Vries A. 2009. Seasonal incidence of lameness and risk factors associated with thin soles, white line disease, ulcers, and sole punctures in dairy cattle. Journal of Dairy Science 92:3165-3174.

Santos LV, Brügemann K, Ebinghaus A, König S. 2018. Genetic parameters for longitudinal behaviour and health indicator traits generated in automatic milking systems. Archives Animal Breeding 61:161-171.

Sasson G, Ben-Shabat SK, Seroussi E, Doron-Faigenboim A, Shterzer N, Yaacoby S, Berg Miller ME, White BA, Halperin E, Mizrahi I. 2017. Heritable bovine rumen bacteria are phylogenetically related and correlated with the cow's capacity to harvest energy from its feed. mBio 8:e00703.

Schöpke K, Gomez A, Dunbar KA, Swalve HH, Döpfer D. 2015. Investigating the genetic background of bovine digital dermatitis using improved definitions of clinical status. Journal of Dairy Science 98:8164-8174.

Sorensen LP, Mark T, Madsen P, Lund MS. 2009. Genetic correlations between pathogen-specific mastitis and somatic cell count in Danish Holsteins. Journal of Dairy Science 92:3457-3471.

Soyeurt H, Colinet FG, Arnould VM-R, Dardenne P, Bertozzi C, Renaville R, Portetelle D, Gengler N. 2007a. Genetic variability of lactoferrin content estimated by mid-infrared spectrometry in bovine milk. Journal of Dairy Science 90:4443-4450.

Soyeurt H, Gillon A, Vanderick S, Mayeres P, Bertozzi C, Gengler N. 2007b. Estimation of heritability and genetic correlations for the major fatty acids in bovine milk. Journal of Dairy Science 90:4435-4442.

Soyeurt H, Dehareng F, Mayeres P, Bertozzi C, Gengler N. 2008. Genetic parameters of saturated and monounsaturated fatty acid content and the ratio of saturated to unsaturated fatty acids in bovine milk. Journal of Dairy Science 91:3611-3626.

Soyeurt H, Dehareng F, Romnée JM, Gengler N, Dardenne. 2012. Genetics of the mineral contents in bovine milk predicted by mid-infrared spectrometry. 63rd Annual Meeting of the Association of European Animal Production, 27 August, Bratislava, Slovakia. Retrieved 08 March 2018, from https://orbi.uliege.be/bitstream/2268/129984/1/Session 03_Soyeurt.pdf.

Stoop WM, van Arendonk JAM, Heck JML, van Valenberg HJF, Bovenhuis H. 2008. Genetic parameters for major milk fatty acids and milk production traits of Dutch Holstein-Friesians. Journal of Dairy Science 91:385-394.

Streit M, Reinhardt F, Thaller G, Bennewitz J. 2012. Reaction norms and genotype-by-environment interaction in the German Holstein dairy cattle. Journal Animal Breeding Genetics 129:380–389.

Tajima K, Nonaka I, Higuchi K, Takusari N, Kurihara M, Takenaka A, Mitsumori M, Kailkawa H, Aminov R. 2007. Influence of high temperature and humidity on rumen bacterial diversity in Holstein heifers. Anaerobe 13:57-64.

Tetens J, Heuer C, Heyer I, Klein MS, GronwaldW, Junge W, Oefner PJ, Thaller G, Krattenmacher N. 2015. Polymorphisms within the *APOBR* gene are highly associated with milk levels of prognostic ketosis biomarkers in dairy cattle. Physiological Genomics 47:129-137.

Thompson-Crispi KA, Sewalem A, Miglior F, Mallard BA. 2012. Genetic parameters of adaptive immune response traits in Canadian Holsteins. Journal of Dairy Science 95:401-409.

Thompson-Crispi KA, Miglior F, Mallard BA. 2013. Genetic parameters for natural antibodies and associations with specific antibody and mastitis in Canadian Holsteins. Journal of Dairy Science 96:3965-3972.

Twomey AJ, Graham DA, Doherty ML, Blom A, Berry DP. 2018a. Little genetic variability in resilience among cattle exists for a range of performance traits across herds in Ireland differing in *Fasciola hepatica* prevalence. Journal of Animal Science 96:2099-2112.

Twomey AJ, Carroll RI, Doherty ML, Byrne N, Graham DA, Sayers RG, Blom A, Berry DP. 2018b. Genetic correlations between endo-parasite phenotypes and economically important traits in dairy and beef cattle. Journal of Animal Science 96:407-421.

van der Drift SGA, van Hulzen KJE, Tweldemedhn TG, Jorritsma R, Nielen M, Heuven HCM. 2012. Genetic and nongenetic variation in plasma and milk ß-hydroxybutyrate and milk acetone concentrations of early-lactation dairy cows. Journal of Dairy Science 95:6781-6787.

Van Haelst YNT, Beeckman A, van Knegsel ATM, Fievez V. 2008. Short Communication: Elevated concentrations of oleic acid and long-chain fatty acids in milk fat of multiparous subclinical ketotic cows. Journal of Dairy Science 91:4683-4686.

van Hulzen KJE, Sprong RC, van der Meer R, van Arendonk JAM. 2009. Genetic and nongenetic variation in concentration of selenium, calcium, potassium, zinc, magnesium, and phosphorus in milk of Dutch Holstein-Freisian cows. Journal of Dairy Science 92:5754-5759.

Wagter LC, Mallard BA, Wilkie BN, Leslie KE, Boettcher PJ, Dekkers JCM. 2000. A quantitative approach to classifying Holstein cows based on antibody responsiveness and its relationship to peripartum mastitis occurrence. Journal of Dairy Science 83:488-498.

Walther S, Tietze M, Czerny CP, König S, Diesterbeck US. 2016. Development of a Bioinformatics Framework for the detection of gene conversation and the analysis of combinatorial diversity in immunoglobulin heavy chains in four cattle breeds. PloS One 11:e0164567.

Weigel KA, Rekaya R. 2000. A multiple-trait herd cluster model for international dairy sire evaluation. Journal of Dairy Science 83:815-821.

West JW 2003. Effects of heat-stress on production in dairy cattle. Journal of Dairy Science 86:2131-2144.

Wheelock JB, Rhoads RP, VanBaale MJ, Sanders SR, Baumgard LH. 2010. Effects of heat stress on engergetic metabolism in lactating Holstein cows. Journal of Dairy Science 93:644-655.

Wood GM, Boettcher PJ, Kelton DF, Jansen GB. 2004. Phenotypic and genetic influences on test-day measures of acetone concentration in milk. Journal of Dairy Science 87:1108-1114.

Zwald NR, Weigel KA, Chang YM, Welper RD, Clay JS. 2004. Genetic selection for health traits using producer-recorded data I. Incidence rates, heritability estimates, and sire breeding values. Journal of Dairy Science 87:4287-4294.

KAPITEL 3

Genome-wide associations and functional gene analyses for endoparasite resistance in an endangered population of native German Black Pied cattle

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Abstract

Gastrointestinal nematodes (GIN), liver flukes (*Fasciola hepatica*) and bovine lungworms (*Dictyocaulus viviparus*) are the most important parasitic agents in pastured dairy cattle. Endoparasite infections are associated with reduced milk production and detrimental impacts on female fertility, contributing to economic losses in affected farms. In quantitative genetic studies, the heritabilities for GIN and *F. hepatica* were moderate, encouraging studies on genomic scales. Genome-wide association studies (GWAS) based on dense single nucleotide polymorphism (SNP) marker panels allow exploration of the underlying genomic architecture of complex disease traits. The current GWAS combined the identification of potential candidate genes with pathway analyses to obtain deeper insights into bovine immune response and the mechanisms of resistance against endoparasite infections.

A 2-step approach was applied to infer genome-wide associations in an endangered dual-purpose cattle subpopulation [Deutsches Schwarzbuntes Niederungsrind (DSN)] with a limited number of phenotypic records. First, endoparasite traits from a population of 1166 Black and White dairy cows [including Holstein Friesian (HF) and DSN] naturally infected with GIN, *F. hepatica* and *D. viviparus* were precorrected for fixed effects using linear mixed models. Afterwards, the precorrected phenotypes were the dependent traits (rFEC-GIN, rFEC-FH, and rFLC-DV) in GWAS based on 423,654 SNPs from 148 DSN cows. We identified 44 SNPs above the genome-wide significance threshold (p_{Bonf} = 4.47 x 10^{-7}), and 145 associations surpassed the chromosome-wide significance threshold (range: 7.47 x 10^{-6} on BTA 1 to 2.18 x 10^{-5} on BTA 28). The associated SNPs identified were annotated to 23 candidate genes. The DAVID analysis inferred four pathways as being related to immune response mechanisms or involved in host-parasite interactions. SNP effect correlations considering specific chromosome segments indicate that breeding for resistance to GIN or *F. hepatica* as measured by fecal egg counts is genetically associated with a higher risk for udder infections.

We detected a large number of loci with small to moderate effects for endoparasite resistance. The potential candidate genes regulating resistance identified were pathogen-specific. Genetic antagonistic associations between disease resistance and productivity were specific for specific chromosome segments. The 2-step approach was a valid methodological approach to infer genetic mechanisms in an endangered breed with a limited number of phenotypic records.

Background

Endoparasite infections imply impaired cattle health and increasing economic losses in pasture-based production systems (Holzhauer et al., 2011; Knubben-Schweizer et al., 2010). Gastrointestinal nematodes (GIN), the bovine lungworm (*Dictyocaulus viviparus*) and the liver fluke (*Fasciola hepatica*) are the most important parasitic helminths of pastured dairy cows (Charlier et al., 2014; Vercruysse et al., 2001). Subclinical infections were associated with reduced milk production (Charlier et al., 2005; Mezo et al., 2011), impaired reproductive performance (Charlier et al., 2007) and a decrease in product quality (Charlier et al., 2009).

From a farm management perspective, prophylactic as well as diagnosis-based anthelmintic treatments can be applied to control endoparasite infections in affected dairy herds (Stear et al., 2007; Vercruysse et al., 2001). However, anthelmintic treatments are expensive, and drug residues might pollute the environment and food products (Wall and Strong, 1987). Furthermore, anthelmintic treatments contribute to anthelmintic resistance (Gasbarre, 2014; McMahon et al., 2013; Sutherland et al., 2011). Hence, sustainable endoparasite control implies the consideration of proper breeding and selection strategies (Bisset et al., 2001; Stear et al., 2007). In this regard, breeding approaches have focused on the selection of specific breeds or genetic lines representing enhanced resistance against specific endoparasites (May et al., 2017a; Oliveira et al., 2009).

In cattle, heritability estimates ranged from 0.04 to 0.36 for various definitions of GIN and *D. viviparus* infections [e.g., fecal egg count (FEC), antibody level], indicating a genetic component for pathogen-specific susceptibility (Barlow and Piper, 1985; Burrow, 2001). For the liver fluke *F. hepatica*, the heritabilities ranged from 0.09 to 0.33 (May et al., 2017a; Twomey et al., 2016, 2018). The pronounced additive-genetic variances identified were stimuli to explore the underlying genomic architecture for endoparasite resistance in cattle and sheep, with a focus on GIN (Dominik, 2005; Gasbarre et al., 2001; Liu et al., 2011). Genome-wide association studies (GWAS) using dense single-nucleotide polymorphism (SNP) marker panels and QTL mapping approaches contributed to the identification of candidate genes related to immune mechanisms (e.g., the *IFNγ* gene, major histocompatibility complex (MHC)-related genes) against GIN infections in cattle and sheep (Benavides et al., 2015; Coppieters et al., 2009; Davies et al., 2006; Sayre and Harris, 2001). Coppieters et al. (2009) based their studies on microsatellite mapping in Dutch HF cows, and they identified two genome-wide significant QTL on BTA 9 and on BTA 19 influencing FEC for GIN infections. In an experimental Angus population and using microsatellite markers, genome-wide suggestive QTL on BTA 8 and

potential linkage with segments on BTA 4, 12 and 17 were associated with patent GIN infections (Kim et al., 2014). A GWAS based on 50,000 SNPs identified 12 genomic regions on BTA 3, 5, 8, 15 and 27 as contributing to FEC variation in Angus cattle (Kim et al., 2015). Potential candidate genes were related to immunological pathways, i.e., the toll-like receptor-signaling pathway and the cytokine-cytokine receptor interaction pathway (Kim et al, 2015). In Angus cattle, association studies based on copy number variations (CNV) have identified immune-related genes, i.e., the genes involved in GIN resistance mechanisms (Liu et al., 2011; Hou et al., 2012; Xu et al., 2014). Although infections with the liver fluke *F. hepatica* in dairy cattle represent a serious animal health problem worldwide (Mehmood et al., 2017), studies with a focus on the identification of genomic variants influencing *F. hepatica* resistance are lacking. In sheep, a QTL microsatellite mapping study (Piedrafita et al., 2010) detected QTL for resistance against *Fasciola gigantica* on OAR 10, 13, 17, 18 and 19, 22.

In Europe, the rising importance of maintaining dairy cattle in grassland systems implies exposure to endoparasite infections and further pathogenic agents (Gordon et al., 2013). Thus, there is increasing interest in local breeds being best adapted to harsh environments and being less susceptible against infections. The local dual-purpose German Black Pied cattle (DSN, German: Deutsches Schwarzbuntes Niederungsrind) is the founder breed of the modern Holstein Friesian (HF) cattle, with a long breeding history in the grassland region of East Frisia, Lower Saxony, Germany (Mügge et al., 1999). DSN are an endangered breed because they are not competitive with HF regarding milk and protein yield. DSN are defined as robust cattle under harsh environmental conditions (Al-Kanaan, 2016), and they show better female fertility parameters and a better health status for metabolic disorders after calving compared with HF (Jaeger et al., 2016). Susceptibility to endoparasite infections as measured by the levels of endoparasite burden (i.e., resistance) may not reflect the host's actual ability to limit the impact of endoparasite infections on fitness and production (i.e., tolerance) (Bishop, 2012). Hence, DSN cows with a high FEC for *F. hepatica* and larvae counts for *D. viviparus* had low somatic cell counts (May et al., 2017a). The udder somatic cell count is a commonly used indicator for mastitis and udder health in overall breeding goals (Martin et al., 2018). High levels of somatic cells in milk reflect leukocyte recruitment and indicate udder inflammation. Pimentel et al. (2011) discussed antagonistic associations among functional traits and between functional traits and productivity on quantitative-genetic and phenotypic scales. However, the correlations were partly favorable when only considering specific important chromosome segments. Hence, a deeper understanding of the physiological or biological trait interactions is imperative in order

to infer the antagonistic relationships between resistance against endoparasites and resistance against udder infections.

For small cattle populations, the limited number of records for complex quantitative traits, especially for health traits, is a special challenge in genomic studies (Schöpke and Swalve, 2016). The use of multibreed reference populations to train on data from several breeds simultaneously was suggested to increase genomic prediction accuracies for production traits (Hayes et al., 2009; Karoui et al., 2012). However, it remains challenging to harmonize recording schemes for novel traits across country borders. Within countries, a further methodological approach might be a 2-step strategy. Step 1 involves using a larger data pool of phenotypes from several breeds or genetic lines and correcting the data for fixed effects. Afterwards, in step 2, precorrected phenotypes are dependent traits in GWAS, considering just the small population. A similar strategy was applied to infer quantitative-genetic (co)variance components in small datasets including only daughter records from specific sires (König et al. 2002).

The objectives of the present study were i) to identify genome-wide associations for resistance or susceptibility (measured by FEC) to three endoparasite infections (GIN, *D. viviparus* and *F. hepatica*) in the endangered DSN breed using a 2-step approach; ii) to assess annotations to potential candidate genes and to infer physiological pathways; and iii) to estimate how SNP affects the correlations among endoparasite traits and between endoparasite traits with the test-day milk yield and test-day somatic cell count in chromosomal segments with an impact on disease resistance.

Methods

Animals

The study was incorporated in the framework of a ‘pasture genetics project’ established in 2007 in northwestern Germany. In the framework of this project, a sample of 1166 German Black and White dairy cows distributed over 17 grassland farms was used for genetic line comparisons and quantitative-genetic studies (May et al., 2017a). The five Black and White genetic lines included an HF line selected for milk yield (HF milk); an HF line suited for grazing conditions (HF pasture); a New Zealand HF line (HF NZ); crosses between HF with Jersey, Angler or beef cattle sires (HF cross); and DSN. All cows were exposed to endoparasite infections (access to pasture before 1st of June with > 8 h per day) and not treated with anthelmintics in the sampling year.

A subset of 148 DSN cows from three different farms was selected for genotyping using a selective genotyping approach. In this regard, the selection criteria were i) the herd prevalence for GIN, as GIN was the endoparasite with the highest prevalence in the initial dataset of 1166 cows, ii) individual parasitological measurements per farm (i.e., considering the extreme phenotypes per herd for GIN), and iii) the pedigree-based genetic relationships. The aim was to minimize the average relationship coefficients between all cows selected for genotyping within and between GIN-infected and GIN-non infected cows.

Phenotypes

The endoparasite dataset considered FEC for GIN (FEC-GIN) and *F. hepatica* (FEC-FH) as well as fecal larvae counts (FLC) for *D. viviparus* (FLC-DV). Based on the coproscopical results, the predominant morphotype for GIN was strongylid eggs (Trichostrongylidae or *Oesophagostomum* and *Bunostomum* spp., respectively) followed by *Strongyloides papillosus* and *Capillaria* spp. eggs (see May et al. 2017b). The whole dataset (n = 1166 cows) considered repeated measurements for 840 cows. The endoparasite trait definitions in the laboratory are described by May et al. (2017b). The test-day production traits included repeated measurements from the whole lactation of the sampling year. Cows with less than five test-day records were excluded from the analysis. The somatic cell counts were log-transformed into somatic cell score: SCS = $\log_2$ (SCC/100.000) +3 (Ali and Shook, 1980). Descriptive statistics for the endoparasite traits (FEC-GIN, FEC-FH, FLC-DV) and test-day traits (MY, SCS) for all Black and White cows (whole dataset, n= 1166 cows) and for the genotyped DSN cows (genotype dataset, n = 148) are displayed in Table 1.

Table 1. Descriptive statistics for endoparasite traits for all cows and for genotyped DSN cows.

Endoparasite trait[1]	No. of observations	No. of cows	Mean	SD	Min.	Max.
All cows (n = 1166)						
FEC-GIN	1997	1166	11.35	22.57	0	225.0
FEC-FH	2006	1166	0.61	3.64	0	89.0
FLC-DV	1988	1163	0.17	2.14	0	46.0
MY[2]	10,132	1049	22.06	6.98	2.20	57.20
SCS[3]	10,115	1049	3.02	1.64	0.01	10.01
Genotyped cows (n = 148)						
FEC-GIN	256	148	15.53	29.80	0	225.0
FEC-FH	256	148	0.57	2.06	0	16.0
FLC-DV	255	148	0.56	4.20	0	45.0
MY	1376	148	19.98	5.77	4.30	47.90
SCS	1369	148	3.07	1.51	0.01	8.60

[1]FEC-GIN = fecal egg count for gastrointestinal nematodes; FEC-FH = fecal egg count for *F. hepatica*; FLC-DV = fecal larvae count for *D. viviparus*.

[2]MY = Milk yield (kg/cow/day).

[3]SCS = somatic cell score (log-transformed somatic cell count: $\log_2$ (SCC/100.000) +3).

Genotypes and quality control

The DSN cows were genotyped using the BovineSNP50 Bead Chip V2 (50k SNP chip) following the Illumina Infinium assay protocol (Illumina Inc., San Diego, CA, USA). In the next step, the genotypes were imputed into Illumina HD Bead Chip level (700k SNP chip) using a multibreed reference panel of 2188 animals. The reference panel considered 48 DSN animals genotyped with the Illumina HD 700k Bead Chip array (Illumina Inc., San Diego, CA, USA) and 2140 sequenced animals (including 30 sequenced DSN animals) from the 1000 bull genome project database (Daetwyler et al., 2014) downscaled to Illumina HD Bead Chip density. Imputation was performed using BEAGLE 4.1 software (Browning and Browning, 2016). The average imputation accuracy from a leave-one-out approach (Korkuć et al., 2017) was 89.3%. Only SNP markers on autosomes with validated locations (i.e., based on BLAST analysis against the bovine genome assembly UMD3.1) were considered (Korkuć et al., 2017). The

imputed dataset included 587,615 SNP markers from 148 DSN cows. Quality control of the imputed genotype data was performed using the software package PLINK, version 1.9 (Purcell et al, 2007). SNP markers with a minor-allele frequency (MAF) < 0.05, significant deviation from Hardy-Weinberg equilibrium (HWE, $p < 10^{-6}$) or a call rate < 95% were discarded. Individuals with a call rate < 95% were also excluded. After quality control, the final dataset for GWAS contained 423,654 SNP marker genotypes.

Potential stratification in the dataset due to relatedness among sampled individuals was examined prior to the GWAS using a principal component analysis (PCA). The PCA based on the variance-standardized relationship matrix was derived from the SNP markers as implemented in the --pca option in PLINK.

Statistical models

Precorrection of phenotypic data

In the first step, phenotypes for the endoparasite traits (FEC-GIN, FLC-DV, FEC-FH) and the test-day traits MY and SCS were precorrected for fixed effects using the initial dataset from all 1166 Black and White cows (consideration of all genetic lines). Precorrection of the endoparasite traits for fixed effects was accomplished via a linear mixed model analysis (model 1) using the statistical software SAS, version 9.4 (SAS PROC MIXED, ML method):

$$y_{ijklm} = \mu + F_i + P_j + GL_k + SP_l + LS_m + e_{ijklm} \quad (1)$$

y_{ijklm} = observations for FEC-GIN, FEC-FH and FLC-DV; μ = overall mean effect; F_i = fixed effect of the i^{th} farm (i = 1,…, 17); P_j = fixed effect of the j^{th} parity number (j = 1, 2, 3, 4, > 4); GL_k = fixed effect of the k^{th} genetic line (k = HF milk, HF pasture, HF NZ, DSN, HF cross); SP_l = fixed effect of the l^{th} sampling period (l = June/July, September/October); LS_m = fixed effect of the m^{th} lactation stage according to Huth (1995) (m = ≤ 14 days in milk (DIM), 14 - 77 DIM, 78 - 140 DIM, 141 - 231 DIM, ≥232 DIM); and e_{ijklm} = random residual effect.

Hereafter, the precorrected phenotypes (residuals) for the endoparasite traits (FEC-GIN, FEC-FH, FLC-DV) are denoted as rFEC-GIN, rFEC-FH and rFLC-DV, respectively. Precorrected phenotypes were available from 148 genotyped DSN cows. The distribution of residuals for the endoparasite traits was checked and visually inspected. To further validate the precorrection approach, we correlated the estimated breeding values (EBVs) for all three endoparasite traits from the animal models in May et al. (2017a) with EBVs from animal models based on precorrected phenotypes. For all traits the EBV correlations between models were > 0.95.

Accordingly, the test-day traits were precorrected for fixed effects via linear mixed model applications (model 2) (SAS PROC MIXED, ML method):

$$y_{ijklm} = \mu + HTD_i + P_j + TS_k + YS_l + DIM_m + e_{ijklm} \quad (2)$$

y_{ijklm} = observations for MY and SCS; HTD_i = fixed effect of the i^{th} herd-test-date; P_j = fixed effect of j^{th} parity number (1, 2, 3, 4, >4); TS_k = fixed effect of k^{th} time span between each test-day record and the endoparasite sampling date (≤ -200, >-200 and ≤ -100, >-100 and ≤0, >0 and ≤100, >100); YS_l = fixed effect of l^{th} year-season of last calving (spring, summer, autumn, winter within each year); DIM_m = covariate for days in milk modeled with Legendre polynomials of order 3; and e_{ijklm} = random residual effect. Hereafter, the precorrected phenotypes (residuals) for the test-day MY and SCS are denoted as rMY and rSCS, respectively.

Genome-wide association analyses

In the second step, precorrected phenotypes (i.e., residuals from step 1: rFEC-GIN, rFEC-FH, rFLC-DV, rMY, rSCS) were used as dependent variables in single-trait GWAS as implemented in the software package GCTA (Yang et al., 2011). All association analyses were performed using the --mlma option in GCTA. The following statistical model for testing single-locus SNP effects was applied:

$$\boldsymbol{y} = \mathbf{1}\boldsymbol{\mu} + \boldsymbol{x}\boldsymbol{b} + \boldsymbol{u} + \boldsymbol{e}$$

where $\boldsymbol{y}$ = vector of precorrected phenotypes (rFEC-GIN, rFLC-DV, rFEC-FH, rMY and rSCS); $\boldsymbol{\mu}$ = the overall mean; $\boldsymbol{b}$ = additive fixed effect of the candidate variant tested for association; **x** = vector of genotypes for the candidate SNP; $\boldsymbol{u} \sim N(0, \boldsymbol{G}\sigma_u^2)$ = vector of random polygenic effects; **G** = genomic relationship matrix (GRM); σ_u^2 = polygenic variance estimated from a null model (i.e., $y = 1\mu + u + e$); and $\boldsymbol{e} \sim N(0, \boldsymbol{I}\sigma_e^2)$ = vector of random residual effects, where **I** = an identity matrix and σ_e^2 = the residual variance.

An adjusted Bonferroni correction was applied to account for multiple testing. The traditional Bonferroni correction (i.e., relating the genome-wide significance threshold of 0.05 to the total number of SNP) tends to produce many false-negative results (Wellcome Trust Case Control Consortium, 2007). Therefore, the effective number of independent SNP markers in the analysis (n = 111,901) was estimated based on the LD between markers using the software GEC (Li et al., 2012). The adjusted Bonferroni-corrected genome-wide significance threshold with (p = 0.05 / n) was p_{Bonf} = 4.47 x 10^{-7}. In addition, we considered a chromosome-wide significance threshold (p_{Cand} = 0.05 / n_c), where n_c is the effective number of independent SNP markers of

the respective chromosome. In this regard, we applied GEC (Li et al., 2012)). Chromosome-wide significance thresholds ranged from 7.47×10^{-6} (BTA 1) to 2.18×10^{-5} (BTA 28) (Table 2).

Table 2. Chromosome-wide significance thresholds for the endoparasite traits rFEC-GIN, rFEC-FH and rFLC-DV including the number of SNP markers after quality control (QC) and the effective number of independent SNP markers based on linkage disequilibrium (LD) calculated using the software GEC (Lie et al., 2012).

BTA	SNP marker after QC	Effective number of independent SNP marker	*p*-value threshold p_{Cand}
1	27303	6696	7.47E-06
2	22710	5774	8.66E-06
3	20234	5103	9.80E-06
4	20603	5390	9.28E-06
5	18810	4448	1.12E-05
6	22292	5816	8.60E-06
7	19373	4858	1.03E-05
8	15643	4180	1.20E-05
9	17186	4158	1.20E-05
10	18268	4982	1.00E-05
11	19296	4705	1.06E-05
12	14394	3927	1.27E-05
13	12170	3536	1.41E-05
14	12850	3614	1.38E-05
15	13730	3620	1.38E-05
16	13921	3412	1.47E-05
17	13356	3608	1.39E-05
18	11600	3142	1.59E-05
19	12172	3719	1.34E-05
20	13822	3757	1.33E-05
21	11604	2887	1.73E-05
22	11409	3021	1.65E-05
23	8689	2782	1.80E-05
24	10861	2815	1.77E-05
25	8120	2567	1.95E-05
26	8933	2375	2.11E-05
27	8145	2346	2.13E-05
28	7890	2292	2.18E-05
29	8270	2371	2.11E-05
overall	423654	111902	4.47E-07 (p_{Bonf})

Candidate gene annotation and pathway analyses

The biomaRt package (Durinck et al., 2005, 2009) from the Bioconductor project was applied to retrieve 'rs accession numbers' of associated SNP markers using the getBM() function. Potential candidate genes were queried and assigned to associated SNP markers using the current gene annotations from the ENSEMBL (Version 90) and NCBI (Version 105) databases. A gene was considered as a candidate gene if at least one associated SNP marker above p_{Cand} was positioned i) in the respective gene and/or ii) within 5 kb up- and downstream of the respective candidate gene. Regions including the candidate gene ± 5 kb up- and downstream are hereafter referred to as regions of interest (ROI). The potential candidate genes identified were manually submitted to the DAVID database (Version 6.8) (Huang et al., 2008) for pathway and enrichment analyses. In addition, physiological functions and positions of potential candidate genes were further manually reviewed in the KEGG (Kanehisa et al., 2014), ENSEMBL and NCBI databases.

Calculation of SNP effect correlations between traits

SNP effect correlations were calculated i) among rFEC-GIN, rFEC-FH and rFLC-DV for the respective potential candidate genes for each trait within all identified ROI and ii) within identified ROI for rFEC-GIN, rFEC-FH and rFLC-DV with rMY and rSCS. SNP effects were not correlated for four ROI for rFLC-DV (corresponding genes: *FAM124B*, *ISL2*, *RCN2*, and *SCAPER*) due to the limited number of marker associations (see Table 2) or identical SNP effects within traits (no variance for the respective ROI).

Results

Population stratification

Figure 1-4 include the top two PCs plotted against each other to visualize the population structure with additional color representation for i) individual farm affiliations and ii) individual endoparasite phenotypes. The analysis revealed three main clusters within the whole population caused primarily by the three different farms. Relationships were closer between the individuals of farm 1 (41 cows) and farm 2 (66 cows), whereas the individuals of farm 3 (41 cows) were not closely linked among each other or to farm 1 and farm 2 individuals. Generally, we only found slight stratification induced by kinship. Hence, we did not account for population stratification via the consideration of PCs in the models for GWAS.

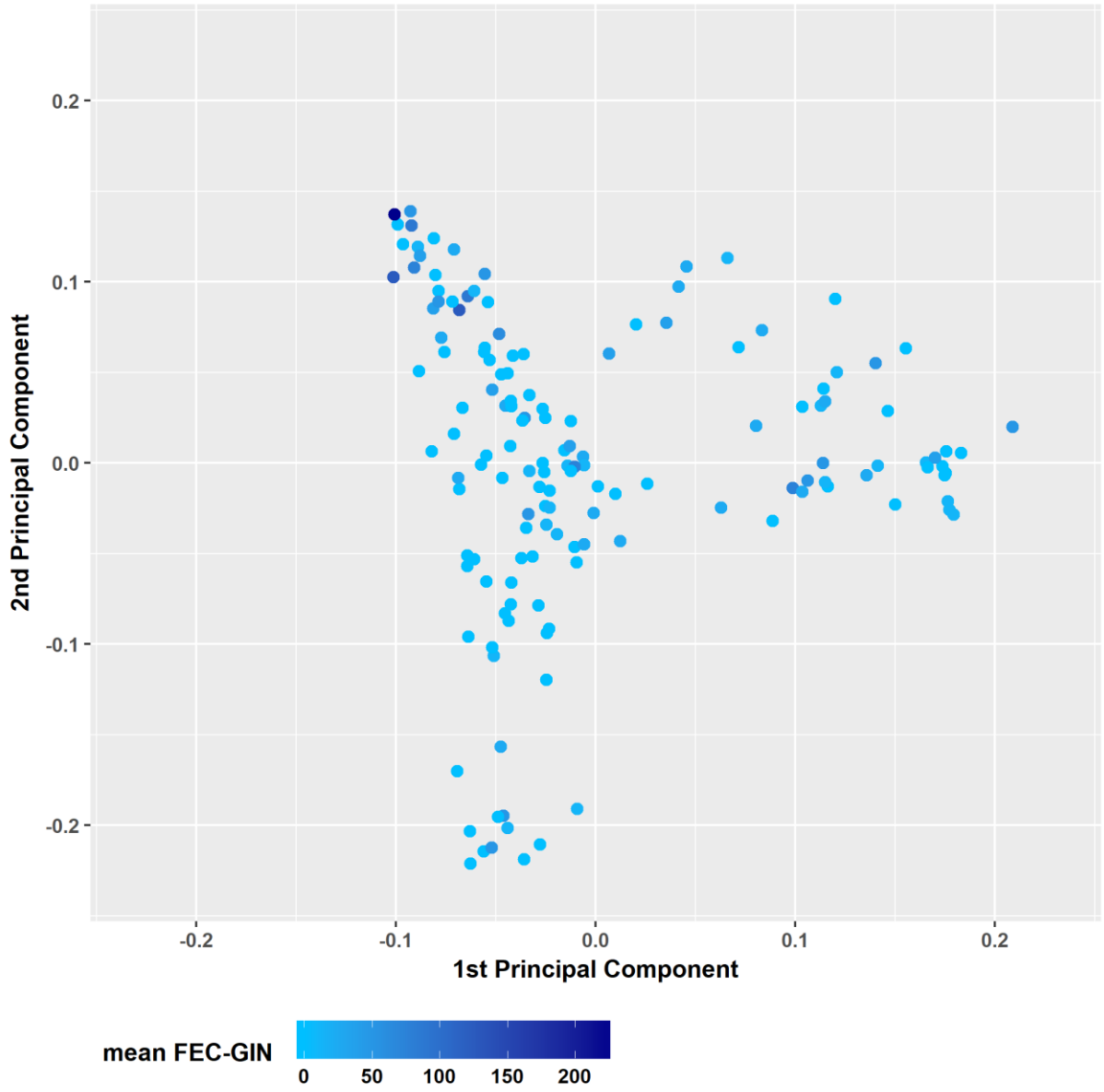

Figure 1. Principal component analysis of the 148 DSN cattle for the mean values of FEC-GIN. Plot of the first two principal components (PC1 and PC2) of each individual cow based on SNP information to evaluate the extent of the population structure.

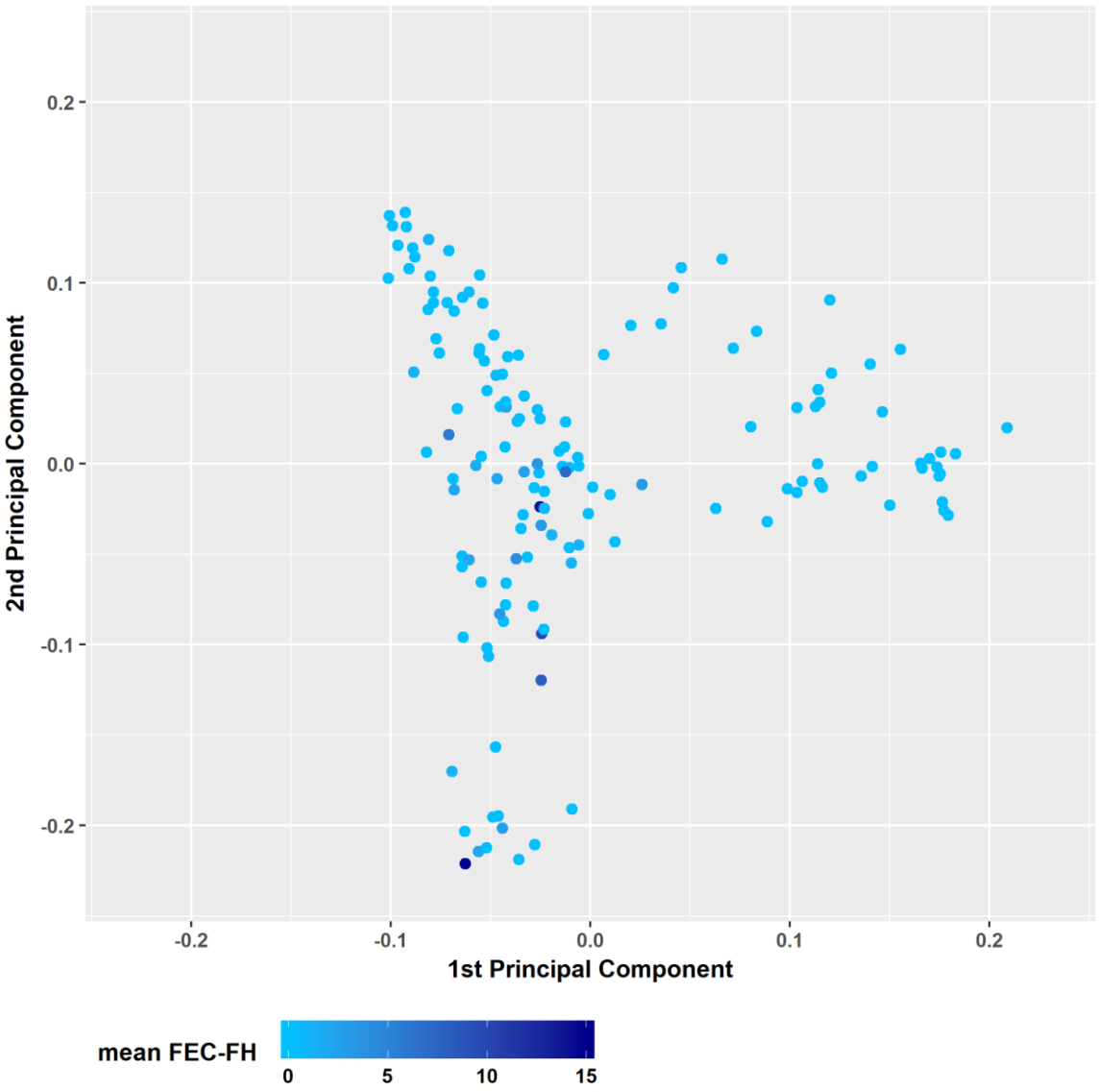

Figure 2. Principal component analysis of the 148 DSN cattle for the mean values of FEC-FH. Plot of the first two principal components (PC1 and PC2) of each individual cow based on SNP information to evaluate the extent of the population structure.

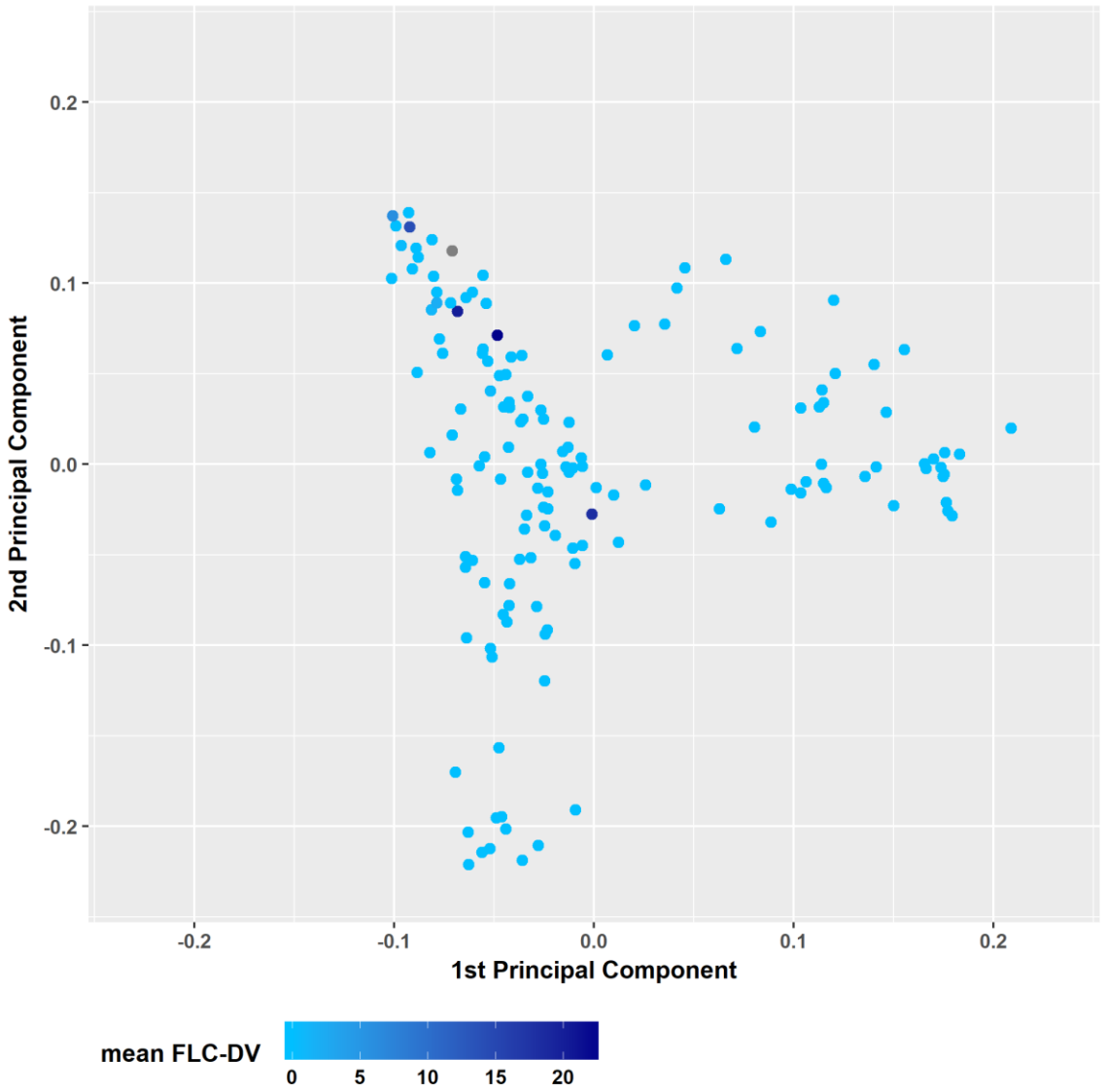

Figure 3. Principal component analysis of the 148 DSN cattle for the mean values of FLC-DV. Plot of the first two principal components (PC1 and PC2) of each individual cow based on SNP information to evaluate the extent of the population structure.

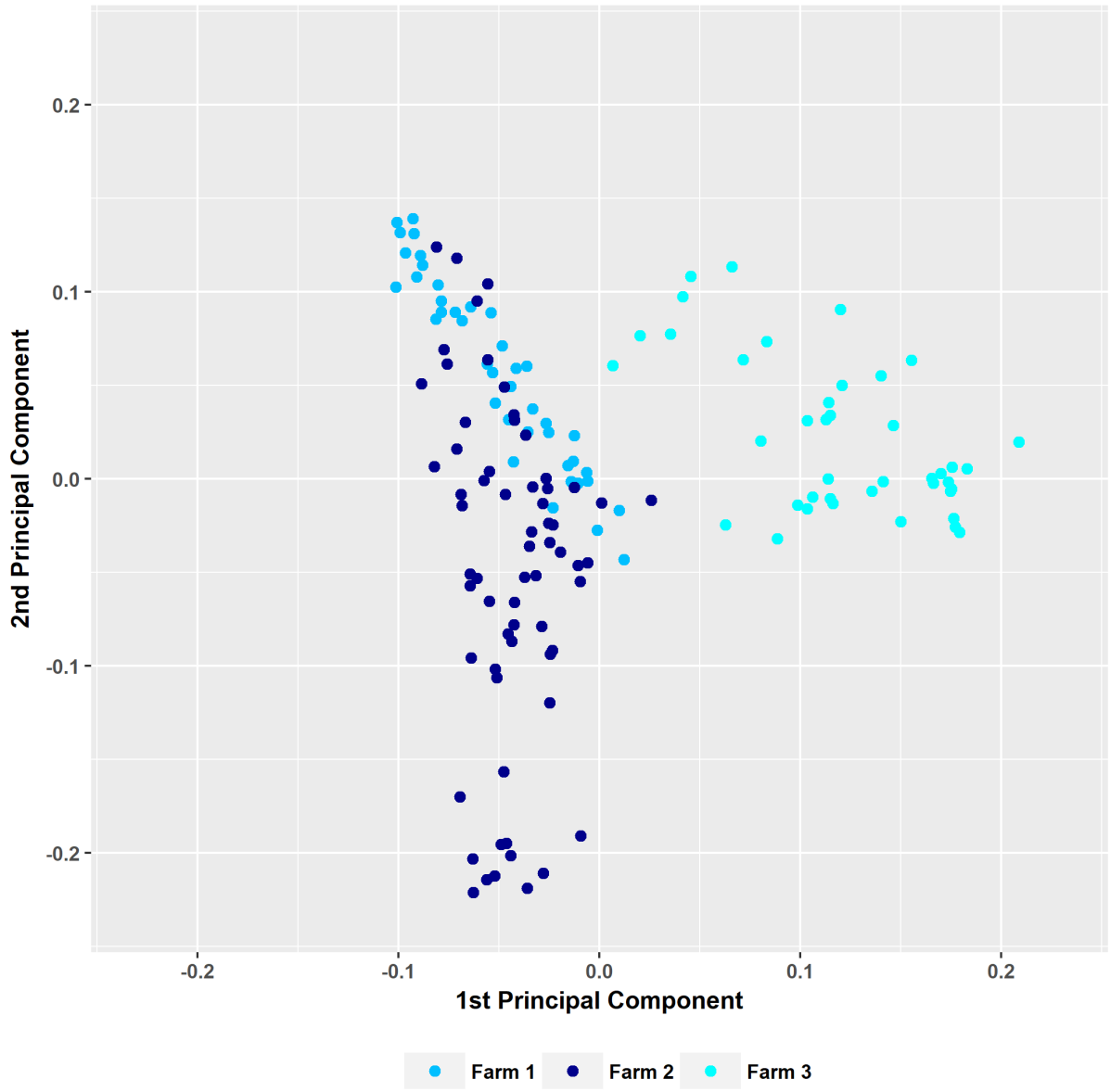

Figure 4. Principal component analysis of the 148 DSN cattle for the three different farms. Plot of the first two principal components (PC1 and PC2) of each individual cow based on SNP information to evaluate the extent of the population structure.

Genome-wide association analysis for endoparasite traits

Manhattan-plots from the GWAS and corresponding Q-Q plots for rFEC-GIN, rFEC-FH and rFLC-DV are given in Figure 5-7. For rFEC-GIN, GWAS identified 17 associated SNP markers based on p_{cand} on 9 chromosomes (Table 3). None of the SNP markers reached the p_{Bonf} level. Most of the associations were detected on BTA 2 (n = 4) and BTA 18 (n = 3). For rFEC-FH, GWAS identified three SNP markers above p_{Bonf} on BTA 7 (Table 4). In total, three additional variants surpassed the suggestive candidate thresholds p_{Cand} on the three chromosomes BTA 1, 7 and 28. GWAS for rFLC-DV identified 41 associations according to the p_{Bonf} threshold on BTA 2, 5, 8, 15, 17, 21 and 24 (Table 5). Moreover, 125 additional variants exceeded the p_{Cand} level with a majority (n = 44) positioned on BTA 29.

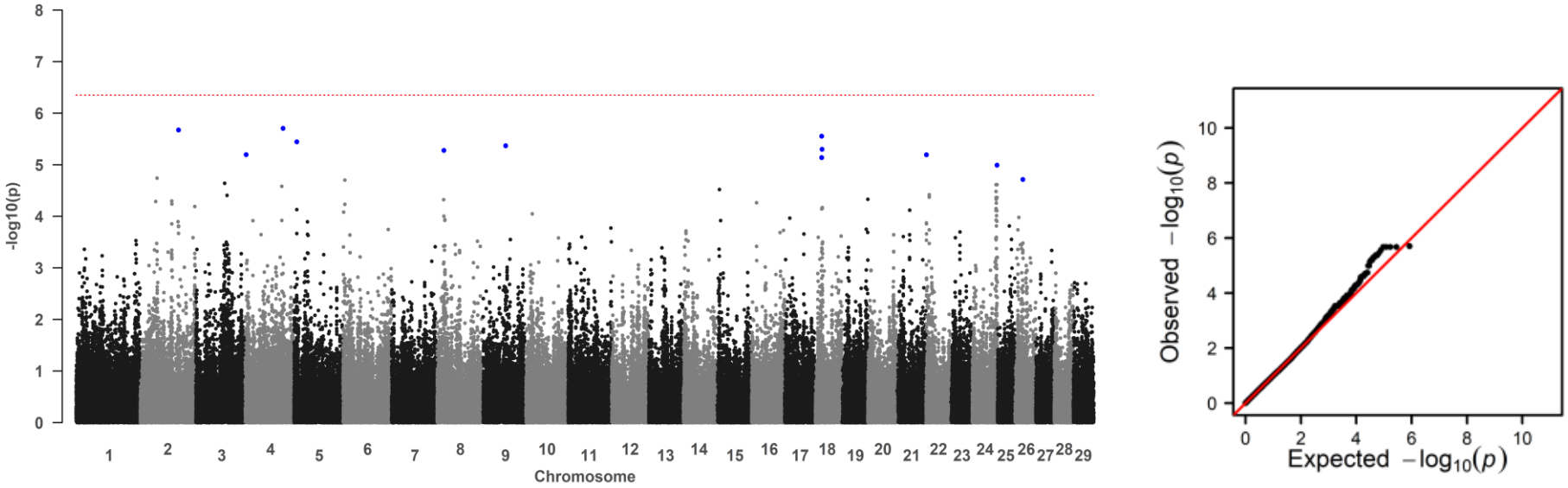

Figure 5. Manhattan plot displaying the GWAS results (*p*-values and corresponding Q-Q plot of observed *p*-values against the expected *p*-values) for rFEC-GIN. Bonferroni-corrected genome-wide significance (red line), SNP marker above p_{Bonf} (marked in red) and SNP marker above suggestive of the chromosome-wide significance threshold (range: $p = 7.47 \times 10^{-6}$ on BTA 1 to $p = 2.18 \times 10^{-5}$ on BTA 28) (marked in blue) are also shown.

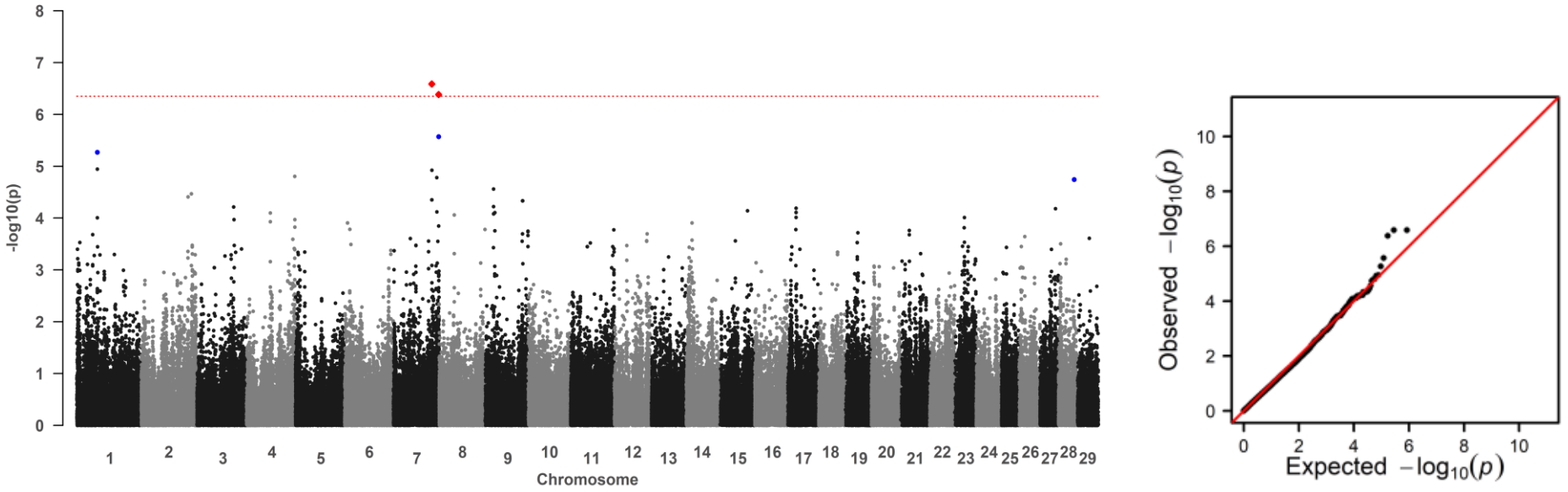

Figure 6. Manhattan plot displaying the GWAS results (*p*-values and corresponding Q-Q plot of observed *p*-values against the expected *p*-values) for rFEC-FH. Bonferroni-corrected genome-wide significance (red line), SNP marker above p_{Bonf} (marked in red) and SNP marker above suggestive of the chromosome-wide significance threshold (range: $p = 7.47 \times 10^{-6}$ on BTA 1 to $p = 2.18 \times 10^{-5}$ on BTA 28) (marked in blue) are also shown.

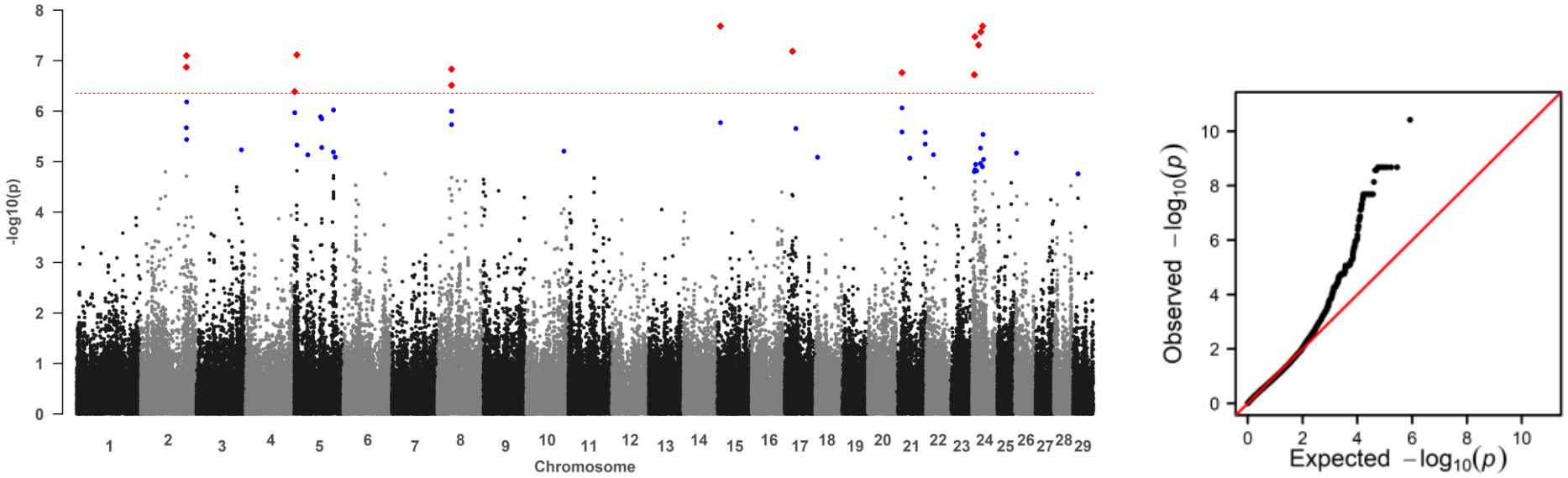

Figure 7. Manhattan plot displaying the GWAS results (*p*-values and corresponding Q-Q plot of observed *p*-values against the expected *p*-values) for rFLC-DV. Bonferroni-corrected genome-wide significance (red line), SNP marker above p_{Bonf} (marked in red) and SNP marker above suggestive of the chromosome-wide significance threshold (range: $p = 7.47 \times 10^{-6}$ on BTA 1 to $p = 2.18 \times 10^{-5}$ on BTA 28) (marked in blue) are also shown.

Table 3. List of all SNP markers associated with the residuals of gastrointestinal nematodes (rFEC-GIN) identified in Black Pied dairy cattle by genome-wide analysis.

BTA	SNP name	Position (bp)	SNP effect	SE	*p*-value
2	*rs135675074*	94,171,783	33.14	6.99	2.12×10^{-6}
	rs132921643	94,172,533	33.14	6.99	2.12×10^{-6}
	rs137012736	94,174,089	33.14	6.99	2.12×10^{-6}
	rs135373903	94,176,421	33.14	6.99	2.12×10^{-6}
4	*rs134978883*	2,906,710	27.15	6.02	6.38×10^{-6}
	rs111009671	94,017,547	32,51	6,83	1.97×10^{-6}
5	*rs43427386*	7,162,997	33.23	7.17	3.59×10^{-6}
8	*rs110654845*	16,726,638	27.17	5.97	5.26×10^{-6}
9	*rs42686248*	55,913,164	21.90	4.76	4.27×10^{-6}
	rs42684203	55,982,484	21.90	4.76	4.27×10^{-6}
18	*rs136760652*	16,111,659	33.08	7.06	2.79×10^{-6}
	rs133189711	16,201,169	29.46	6.57	7.28×10^{-6}
	rs41866588	16,920,014	30.23	6.62	5.02×10^{-6}
22	*rs110780876*	992,265	34.75	7.70	6.41×10^{-6}
24	*rs135792391*	61,565,663	29.51	6.69	1.02×10^{-5}
	rs134104638	61,567,323	29.51	6.69	1.02×10^{-5}
26	*rs42088089*	19,577,461	28.33	6.63	1.93×10^{-5}

Table 4. List of all SNP markers associated with the residuals of *Fasciola hepatica* (rFEC-FH) identified in Black and White dairy cattle by genome-wide analysis.

BTA	SNP name	Position (bp)	SNP effect	SE	*p*-value
1	*rs110835791*	50,365,140	1.56	0.34	5.38×10^{-6}
7	*rs41664083*	94,639,822	1.77	0.34	2.59×10^{-7}
	rs135348628	94,745,593	1.77	0.34	2.59×10^{-7}
	rs136054186	111,644,874	2.28	0.45	4.15×10^{-7}
	rs135096708	111,643,709	2.04	0.43	2.69×10^{-6}
28	*rs133005080*	38,500,927	2.27	0.53	1.81×10^{-5}

Table 5. List of all SNP markers associated with the residuals of *Dictyocaulus viviparus* (rFLC-DV) identified in Black and White dairy cattle by genome-wide analysis.

BTA	SNP name	Position (bp)	SNP effect	SE	*p*-value
2	*rs133101212*	113,205,475	8.92	1.48925	2.10×10^{-9}
	rs43321467	113,207,239	8.92	1.48925	2.10×10^{-9}
	rs43321471	113,210,047	8.92	1.48925	2.10×10^{-9}
	rs133768435	113,210,910	8.92	1.48925	2.10×10^{-9}
	rs135251342	113,213,058	8.92	1.48925	2.10×10^{-9}
	rs43321517	113,223,952	8.92	1.48925	2.10×10^{-9}
	rs43323482	113,291,934	8.92	1.48925	2.10×10^{-9}
	rs43323483	113,293,169	7.28	1.35637	7.95×10^{-8}
	rs41645202	112,993,253	7.04	1.33595	1.35×10^{-7}
	rs134650179	113,478,686	6.34	1.27459	6.59×10^{-7}
	rs110218139	113,024,164	4.00	0.843492	2.14×10^{-6}
	rs133748186	113,486,143	5.82	1.25756	3.66×10^{-6}
3	*rs43365726*	112,096,061	5.90	1.30207	5.84×10^{-6}
	rs43366333	112,118,311	5.90	1.30207	5.84×10^{-6}
	rs43366338	112,124,938	5.90	1.30207	5.84×10^{-6}
5	*rs43422549*	6,916,388	4.40	0.819554	7.69×10^{-8}
	rs42020386	1,736,633	4.56	0.900503	4.12×10^{-7}
	rs136770833	97,265,477	5.10	1.04053	9.46×10^{-7}
	rs133601179	1,562,117	7.03	1.44225	1.07×10^{-6}
	rs137416358	1,566,129	7.03	1.44225	1.07×10^{-6}
	rs133958808	1,572,027	7.03	1.44225	1.07×10^{-6}
	rs133844828	65,424,614	5.81	1.19985	1.29×10^{-6}
	rs110472459	68,420,453	4.60	0.953276	1.42×10^{-6}
	rs137016919	6,914,668	3.32	0.724193	4.69×10^{-6}
	rs109593883	68,355,562	5.40	1.18516	5.28×10^{-6}
	rs109627293	68,366,616	5.40	1.18516	5.28×10^{-6}
	rs134847963	68,374,385	5.40	1.18516	5.28×10^{-6}
	rs109729704	68,383,243	5.40	1.18516	5.28×10^{-6}
	rs133229007	97,105,569	3.60	0.797513	6.46×10^{-6}

BTA	SNP name	Position (bp)	SNP effect	SE	*p*-value
	rs136683052	33,889,721	5.53	1.23422	7.33×10^{-6}
	rs135121769	101,571,894	4.50	1.0082	8.13×10^{-6}
	rs134744846	101,585,766	4.50	1.0082	8.13×10^{-6}
	rs137233679	101,596,166	4.50	1.01	8.13×10^{-6}
	rs136997456	101,618,256	4.50	1.01	8.13×10^{-6}
8	*rs136262795*	35,573,747	7.69	1.46	1.48×10^{-7}
	rs137043944	35,553,300	7.27	1.42	3.08×10^{-7}
	rs41850891	35,564,838	7.27	1.42	3.08×10^{-7}
	rs41850898	35,568,857	7.27	1.42	3.08×10^{-7}
	rs136155930	35,494,872	6.81	1.39	1.00×10^{-6}
	rs137383802	35,597,508	6.81	1.39	1.00×10^{-6}
	rs134324239	35,604,477	6.81	1.39	1.00×10^{-6}
	rs132758433	35,550,656	6.33	1.33	1.84×10^{-6}
	rs110106657	35,565,838	6.33	1.33	1.84×10^{-6}
10	*rs134196410*	94,003,818	6.39	1.41	6.20×10^{-6}
15	*rs41750400*	8,975,191	7.63	1.36	2.06×10^{-8}
	rs41750406	8,979,052	7.63	1.36	2.06×10^{-8}
	rs41750419	8,984,238	7.63	1.36	2.06×10^{-8}
	rs134665586	8,984,833	7.63	1.36	2.06×10^{-8}
	rs41750421	8,985,944	7.63	1.36	2.06×10^{-8}
	rs41750428	8,986,630	7.63	1.36	2.06×10^{-8}
	rs41751433	8,987,788	7.63	1.36	2.06×10^{-8}
	rs41751439	8,988,625	7.63	1.36	2.06×10^{-8}
	rs41751444	8,990,016	7.63	1.36	2.06×10^{-8}
	rs41751452	8,992,789	7.63	1.36	2.06×10^{-8}
	rs109581724	9,025,678	7.63	1.36	2.06×10^{-8}
	rs41749574	9,033,366	7.63	1.36	2.06×10^{-8}
	rs41749567	9,035,157	7.63	1.36	2.06×10^{-8}
	rs110430504	9,037,212	7.63	1.36	2.06×10^{-8}
	rs110968108	8,925,026	6.65	1.39	1.70×10^{-6}
	rs110517446	8,925,594	6.65	1.39	1.70×10^{-6}

BTA	SNP name	Position (bp)	SNP effect	SE	*p*-value
17	*rs109427713*	19,991,734	7.37	1.36	6.53×10^{-8}
	rs29013623	28,168,540	6.35	1.34	2.21×10^{-6}
18	*rs41570068*	6,621,405	5.16	1.16	8.20×10^{-6}
21	*rs41639420*	12,918,304	7.64	1.46	1.73×10^{-7}
	rs110352059	12,793,997	6.81	1.38	8.66×10^{-7}
	rs109220319	12,908,299	5.13	1.09	2.59×10^{-6}
	rs136550449	70,243,037	6.09	1.30	2.62×10^{-6}
	rs134168598	70,244,504	5.82	1.27	4.49×10^{-6}
	rs110352830	32,113,699	6.04	1.36	8.56×10^{-6}
	rs134797073	321,19,934	6.04	1.36	8.56×10^{-6}
	rs135714468	32,123,869	6.04	1.36	8.56×10^{-6}
	rs136760499	32,127,160	6.04	1.36	8.56×10^{-6}
	rs133361420	32,140,037	6.04	1.36	8.56×10^{-6}
	rs133525823	32,160,272	6.04	1.36	8.56×10^{-6}
	rs134517799	32,180,070	6.04	1.36	8.56×10^{-6}
	rs137832357	32,184,897	6.04	1.36	8.56×10^{-6}
	rs135757504	32,203,110	6.04	1.36	8.56×10^{-6}
	rs133802702	32,212,044	6.04	1.36	8.56×10^{-6}
	rs137499414	32,214,988	6.04	1.36	8.56×10^{-6}
	rs134322129	32,243,761	6.04	1.36	8.56×10^{-6}
	rs135506397	32,248,881	6.04	1.36	8.56×10^{-6}
	rs134509449	32,257,369	6.04	1.36	8.56×10^{-6}
	rs137664985	32,260,181	6.04	1.36	8.56×10^{-6}
	rs137266496	32,266,753	6.04	1.36	8.56×10^{-6}
	rs136416616	32,277,971	6.04	1.36	8.56×10^{-6}
	rs133785424	32,285,046	6.04	1.36	8.56×10^{-6}
	rs136698863	32,307,624	6.04	1.36	8.56×10^{-6}
	rs137442229	32,317,793	6.04	1.36	8.56×10^{-6}
	rs136863366	32,355,723	6.04	1.36	8.56×10^{-6}
	rs133438590	32,383,742	6.04	1.36	8.56×10^{-6}
	rs111011829	32,419,140	6.04	1.36	8.56×10^{-6}

BTA	SNP name	Position (bp)	SNP effect	SE	*p*-value
	rs134601812	32,462,382	6.04	1.36	8.56×10^{-6}
	rs133284553	32,484,660	6.04	1.36	8.56×10^{-6}
	rs132774917	32,551,098	6.04	1.36	8.56×10^{-6}
	rs137048883	32,552,338	6.04	1.36	8.56×10^{-6}
	rs135901867	32,553,982	6.04	1.36	8.56×10^{-6}
	rs134638377	32,556,030	6.04	1.36	8.56×10^{-6}
	rs41974882	32,580,916	6.04	1.36	8.56×10^{-6}
	rs110780898	32,589,381	6.04	1.36	8.56×10^{-6}
	rs41974889	32,602,384	6.04	1.36	8.56×10^{-6}
22	*rs110949408*	19,159,796	6.55	1.46	7.31×10^{-6}
24	*rs132977752*	9,694,150	7.96	1.20	3.77×10^{-11}
	rs134111269	8,077,784	5.30	0.89	2.71×10^{-9}
	rs133375229	8,089,513	5.30	0.89	2.71×10^{-9}
	rs137322379	8,118,143	5.79	1.00	7.34×10^{-9}
	rs110828071	26,974,278	7.06	1.26	2.07×10^{-8}
	rs109177598	22,360,773	5.41	0.97	2.70×10^{-8}
	rs41645744	8,071,750	4.64	0.84	3.36×10^{-8}
	rs137780857	16,998,232	5.94	1.09	4.89×10^{-8}
	rs136886816	17,021,421	5.94	1.09	4.89×10^{-8}
	rs133867370	6,336,382	6.54	1.26	1.90×10^{-7}
	rs134267948	27,709,482	6.64	1.42	2.89×10^{-6}
	rs108957431	21,676,840	4.71	1.04	5.41×10^{-6}
	rs111023415	21,681,981	4.71	1.04	5.41×10^{-6}
	rs137002683	29,087,857	3.70	0.83	9.04×10^{-6}
	rs137823178	21,629,240	4.28	0.97	1.09×10^{-5}
	rs109096413	9,652,945	6.50	1.48	1.13×10^{-5}
	rs110649362	26,510,275	5.71	1.31	1.26×10^{-5}
	rs136230042	8,122,526	5.64	1.30	1.45×10^{-5}
	rs109900196	11,953,955	5.36	1.24	1.53×10^{-5}
	rs109345412	6,182,494	5.79	1.34	1.59×10^{-5}
26	*rs109240510*	4,781,510	5.73	1.27	6.77×10^{-6}

BTA	SNP name	Position (bp)	SNP effect	SE	*p*-value
29	*rs133088719*	13,252,956	6.34	1.48	1.75×10^{-5}
	rs136992575	13,254,130	6.34	1.48	1.75×10^{-5}
	rs109000169	132,55,568	6.34	1.48	1.75×10^{-5}
	rs109728884	13,257,135	6.34	1.48	1.75×10^{-5}
	rs137550236	13,268,747	6.34	1.48	1.75×10^{-5}
	rs110061751	13,273,614	6.34	1.48	1.75×10^{-5}
	rs110294632	13,274,592	6.34	1.48	1.75×10^{-5}
	rs136134712	13,276,754	6.34	1.48	1.75×10^{-5}
	rs110849642	13,277,661	6.34	1.48	1.75×10^{-5}
	rs109056182	13,279,443	6.34	1.48	1.75×10^{-5}
	rs133909880	13,285,311	6.34	1.48	1.75×10^{-5}
	rs137446998	13,286,538	6.34	1.48	1.75×10^{-5}
	rs109702906	13,288,322	6.34	1.48	1.75×10^{-5}
	rs110348590	13,293,156	6.34	1.48	1.75×10^{-5}
	rs110428552	13,307,214	6.34	1.48	1.75×10^{-5}
	rs109978220	13,311,063	6.34	1.48	1.75×10^{-5}
	rs109888653	13,329,371	6.34	1.48	1.75×10^{-5}
	rs109737439	13,330,150	6.34	1.48	1.75×10^{-5}
	rs109396565	13,332,265	6.34	1.48	1.75×10^{-5}
	rs108986441	13,333,756	6.34	1.48	1.75×10^{-5}
	rs110498051	13,335,055	6.34	1.48	1.75×10^{-5}
	rs134834406	13,335,947	6.34	1.48	1.75×10^{-5}
	rs110708167	13,339,550	6.34	1.48	1.75×10^{-5}
	rs109690005	13,342,301	6.34	1.48	1.75×10^{-5}
	rs109863863	13,343,481	6.34	1.48	1.75×10^{-5}
	rs110168180	13,346,362	6.34	1.48	1.75×10^{-5}
	rs132779127	13,351,796	6.34	1.48	1.75×10^{-5}
	rs110080951	13,361,612	6.34	1.48	1.75×10^{-5}
	rs109562572	13,370,202	6.34	1.48	1.75×10^{-5}
	rs110243030	13,371,914	6.34	1.48	1.75×10^{-5}
	rs110760342	13,379,969	6.34	1.48	1.75×10^{-5}

BTA	SNP name	Position (bp)	SNP effect	SE	*p*-value
	rs135432702	13,388,874	6.34	1.48	1.75×10^{-5}
	rs41621467	13,392,044	6.34	1.48	1.75×10^{-5}
	rs109167255	13,393,654	6.34	1.48	1.75×10^{-5}
	rs110816500	13,401,385	6.34	1.48	1.75×10^{-5}
	rs136184785	13,414,522	6.34	1.48	1.75×10^{-5}
	rs109526288	13,439,573	6.34	1.48	1.75×10^{-5}
	rs133454249	13,443,333	6.34	1.48	1.75×10^{-5}
	rs109611361	13,456,917	6.34	1.48	1.75×10^{-5}
	rs42163709	13,509,419	6.34	1.48	1.75×10^{-5}
	rs109048330	13,553,384	6.34	1.48	1.75×10^{-5}
	rs133875991	13,559,340	6.34	1.48	1.75×10^{-5}
	rs110349107	13,560,884	6.34	1.48	1.75×10^{-5}
	rs132633317	13,562,508	6.34	1.48	1.75×10^{-5}

Table 6. Potential candidate genes related to the identified regions associated with endoparasite resistance traits.

BTA	Position[1]	No. of associations (total no. of SNP markers)[2]	Position of maximum association (*P*-value)	Gene symbol	Reference [3]
rFEC-GIN					
4	93,956,587 – 94,148,561 §	1 (33)	94,017,547 (1.97 x 10^{-6}) *	*AHCYL2*	ENSBTAG00000001754 | 532836
5	6,777,101 – 7,678,220 §	1 (197)	7,162,997 (3.59 x 10^{-6}) *	*NAV3*	ENSBTAG00000009852 | 528870
18	15,929,200 – 16,156,356 §	1 (10)	16,111,659 (2.79 x 10^{-6}) *	*PHKB*	ENSBTAG00000004806 | 511783
22	890,106 – 1,107,725 §	1 (25)	992,265 (6.41 x 10^{-6}) *	*EGFR*	ENSBTAG00000011628 | 407217
24	61,568,972 – 61,792,092 §	2 (53)	61,565,663 (1.02 x 10^{-5}) *#	*PHLPP1*	ENSBTAG00000045832 | 615982
rFEC-FH					
1	50,331,575 – 50,562,844 §	1 (54)	50,365,140 (5.38 x 10^{-6}) *	*ALCAM*	ENSBTAG00000000088 | 281614
rFLC-DV					
2	113,274,587 – 113,287,683 §	1 (2)	113,291,934 (2.10 x 10^{-9}) **	*FAM124B*	ENSBTAG00000038700 | 506367
	113,369,961 – 113,487,279 §	2 (23)	113,478,686 (6.59 x 10^{-7}) *	*CUL3*	ENSBTAG00000021769 | 534325
5	1,524,095 – 1,565,010 §	3 (13)	1,562,117(1.07 x 10^{-6}) *#	*TPH2*	ENSBTAG00000020792 | 100336620
	6,777,101 – 7,678,220 §	2 (197)	6,916,388 (7.69 x $10^{-8)}$ **	*NAV3*	ENSBTAG00000009852 | 528870
	65,386,934 – 65,454,111 §	1 (4)	65,424,614 (1.29 x 10^{-6}) *	*SLC5A8*	ENSBTAG00000011525 | 615734
	68,385,645 – 68,649,661 §	2 (72)	68,420,453 (1.42 x 10^{-6}) *	*CHST11*	ENSBTAG00000010644 | 528860

BTA	Position[1]	No. of associations (total no. of SNP markers)[2]	Position of maximum association (*P*-value)	Gene symbol	Reference [3]
rFLC-DV					
	97,079,894 – 97,101,600 §	1 (13)	97,105,569 (6.46 x 10^{-6}) *	*EMP1*	ENSBTAG00000036078 | 786490
	97,262,002 – 97,299,627 §	1 (9)	97,265,477 (9.46 x 10^{-7}) *	*FAM234B*	ENSBTAG00000014322 | 512120
	101,572,243 – 101,708,458 §	4 (17)	101,571,894 (8.13 x 10^{-6}) *#	*RIMKLB*	ENSBTAG00000003291 | 538579
21	12,838,632 – 13,098,198 §	2 (58)	12,918,304 (1.73 x 10^{-7}) **	*MCTP2*	ENSBTAG00000013689 | 532150
	32,107,362 – 32,113,036	1 (1)	32,113,699 (8.56 x 10^{-6}) *	*ISL2*	ENSBTAG00000016651 | 786913
	32,118,844 – 32,548,944 §	24 (26)	32,119,934 (8.56 x 10^{-6}) *#	*SCAPER*	ENSBTAG00000007382 | 100140107
	32,577,840 – 32,595,999 §	2 (2)	32,580,916 (8.56 x 10^{-6}) *#	*RCN2*	ENSBTAG00000015780 | 512717
24	7,684,880 – 8,100,543 §	4 (113)	8,077,784 (2.71 x 10^{-9}) **	*DOK6*	ENSBTAG00000046957 | 100336967
	21,641,932 – 21,691,820 §	2 (8)	21,676,840 (5,41 x 10^{-6}) *#	*GALNT1*	ENSBTAG00000011206 | 104975742
	28,992,666 – 29,241,119	1 (60)	29,087,857 (9.04 x 10^{-6}) *	*CDH2*	ENSBTAG00000021190 | 281062
26	4,520,359 – 5,584,422 §	1 (170)	4,781,510 (6.76 x 10^{-6}) *	*PCDH15*	ENSBTAG00000045905 | 100140108

[1] Gene position (start-end) in NCBI annotation build on assembly UMD 3.1.1 (genes with differing start and/or end positions in NCBI 105 and ENSEMBL 90 are denoted by §); [2] Number of associations that reached the suggestive chromosome-wide significance threshold (p_{Cand}, range: p = 7.47 x 10^{-6} on BTA 1 to p = 2.18 x 10^{-5} on BTA 28) or the Bonferroni-corrected genome-wide significance threshold (p_{Bonf} = 4.47 x 10^{-7}) based on the position of the identified candidate gene ± 5 kb up- and downstream; [3] Ensembl ID | Entrez ID; *above $p_{Cand;}$ **above $p_{Bonf;}$ #Including several marker associations revealing the same p-value, the association bases on the SNP marker with the lowest base pair position.

Table 7. Candidate genes related to pathways potentially associated with endoparasite resistance.

Pathway	Endoparasite trait	Candidate gene (BTA)[1]	Possible association to endoparasite infections
Cell adhesion molecules pathway	rFEC-FH rFLC-DV	ALCAM (BTA1), CDH2 (BTA 24)	Cell adhesion interactions of T cells[2]
Cytokine-cytokine interaction pathway	rFEC-GIN	EGFR (BTA 22)	Intercellular regulation and mobilization of adaptive immune response cells[2]
Estrogen signaling pathway	rFEC-GIN	EGFR (BTA 22)	Increase in reproduction rate of parasites as a result of increasing metabolism of 17-ß-estradiol in its host
PI3K-Akt signaling pathway	rFEC-GIN	EGFR (BTA 22), PHLPP1 (BTA 24)	Important functions in cellular immune response[2]

[1]Gene ID (chromosomal location); [2]Based on annotation in KEGG pathway; BTA = Bos taurus chromosome; rFEC-GIN = Residuals of fecal egg counts for gastrointestinal nematodes; rFEC-FH = Residuals of fecal egg counts for *Fasciola hepatica*; rFLC-DV = Residuals of fecal larvae counts for *Dictyocaulus viviparus*

Gene annotation and pathway analysis

We identified five potential candidate genes for rFEC-GIN (Table 6). More than two neighboring significantly associated SNP markers without any non-associated marker positioned between them were defined as an association cluster. One association cluster including two variants on BTA 24 was related to the *PHLPP1* (PH domain and leucine rich repeat protein phosphatase 1) gene. The *PHLPP1* gene is a protein coding gene involved in immunological processes (PI3K-Akt signaling pathway; KEGG entry: bta04915), e.g., the regulation of T cell energy (Table 7). Functional annotation from rFEC-GIN candidate genes revealed the estrogen signaling pathway (KEGG entry: bta04915) for the candidate gene *EGFR* (Table 7). We identified three immunological pathways for the *EGFR* (Epidermal growth factor receptor) gene, with the regulating cells involved in innate as well as adaptive inflammatory host defenses (Table 7). The *ALCAM* (Activated leukocyte cell adhesion molecule) gene was related to rFEC-FH on BTA 1 (Table 6). This gene was annotated to the (immune) cell adhesion molecules (CAMs) pathway (KEGG entry: bta04514) (Table 3).

We detected 17 potential candidate genes for rFLC-DV (Table 2). An association cluster including 24 consecutively positioned and associated SNP markers was detected on BTA 21. This cluster is related to the *SCAPER* (S-Phase cyclin A associated protein in the endoplasmic reticulum) gene. The *GSG1* (Germ cell associated 1) gene and the *RIMKLB* (Ribosomal modification protein RimK-like family member B) gene on BTA 5 revealed association clusters, too. Functional annotation from the rFLC-DV candidate genes revealed the cell adhesion molecules (CAMs) pathway for the *CDH2* gene on BTA 24 (Table 7).

The *NAV3* (neuron navigator 3) gene was the only candidate gene associated with more than one trait (rFEC-GIN and rFLC-DV). *NAV3* is involved in the regulation of interleukin 2 production by T cells. However, the marker associations within *NAV3* did not overlap between rFEC-GIN and rFLC-DV. For rFEC-GIN, one SNP marker positioned in the middle of *NAV3* was identified above $p_{\text{Cand.}}$ For rFLC-DV, two SNP markers positioned near the gene start position were significantly associated. The space between both association signals for rFEC-GIN and rFLC-DV in *NAV3* was approximately 235 kb.

SNP effect correlations between endoparasite traits

The number of SNP markers within all identified ROI ranged from 8 to 197. For three of the five ROI for rFEC-GIN, we found antagonistic (negative) SNP effect correlations (-0.32 to -0.69) between rFEC-GIN with rFEC-FH (Figure 8). Only on BTA 5 (6,772,101 – 7,683,220)

and within the ROI on BTA 24 (bp 61,563,972 – 61,797,092) were correlations slightly positive (Figure 8). We detected moderate to high SNP effect correlations (0.34 to 0.87) between rFEC-GIN and rFLC-DV within all ROI for rFEC-GIN. The SNP effect correlation between marker effects for rFEC-FH and rFEC-GIN was negative (-0.17) for the ROI identified for rFEC-FH (Figure 8). For the same ROI, the correlation between SNP effects for rFEC-FH and rFLC-DV was 0.73 (Figure 8). Considering the identified ROI for rFLC-DV, correlations ranged from -0.53 on BTA 24 to 0.99 on BTA 5 between marker effects for rFLC-DV and rFEC-GIN (Figure 8). The correlations between the marker effects for rFLC-DV and rFEC-FH ranged from -0.47 on BTA 5 to 0.99 on BTA 24 (Figure 8). The correlation was 0.78 between the marker effects for rFLC-DV and rFEC-GIN considering the common ROI on BTA 5 (ROI: bp 6,772,101 – 7,683,220; including the *NAV3* gene) (Figure 9).

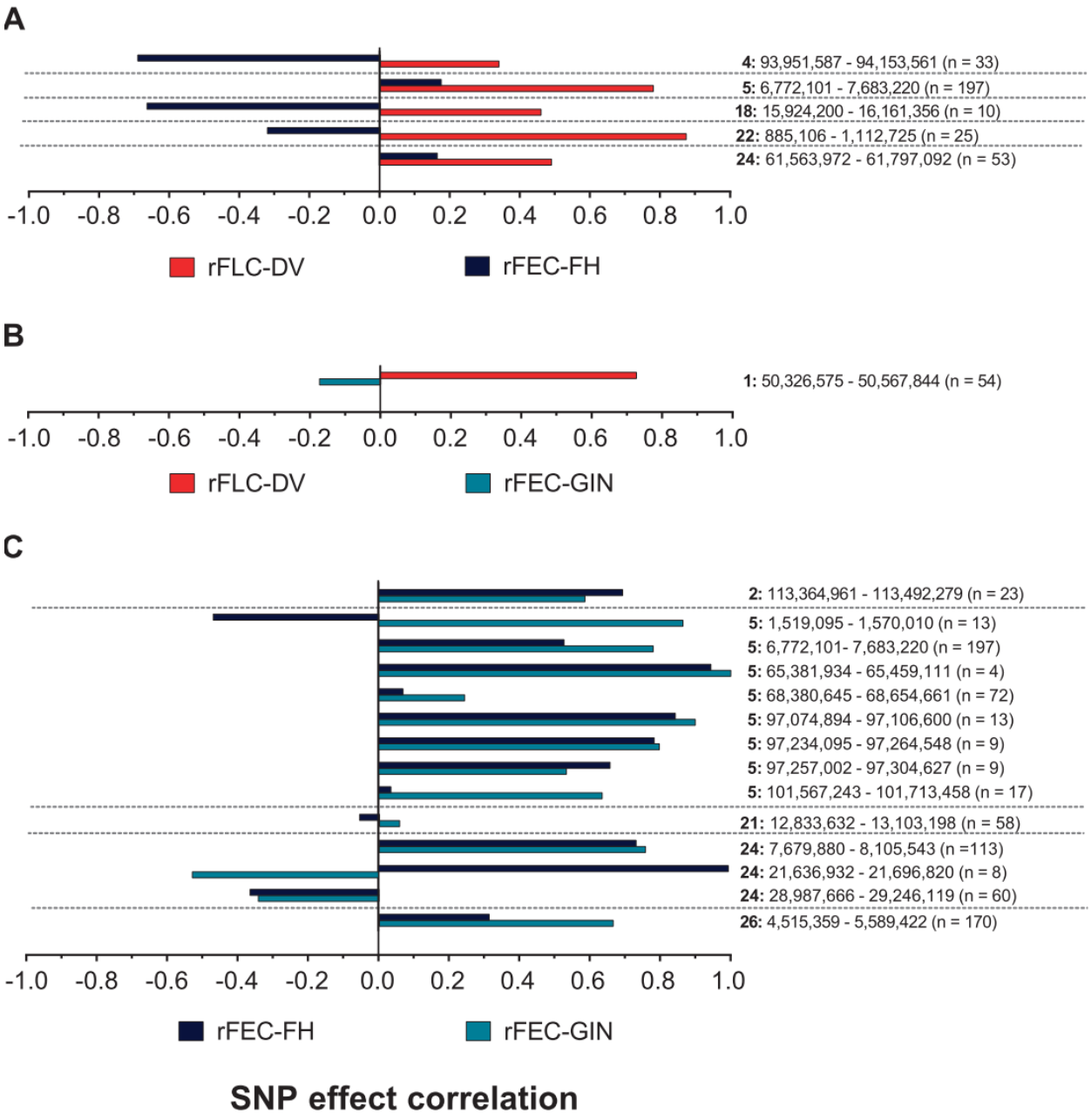

Figure 8. SNP effect correlations between endoparasite traits for the identified genomic regions of potential physiological significance (candidate gene position plus 5 kb up- and downstream) for (A) rFEC-GIN, (B) rFEC-FH and (C) rFLC-DV.

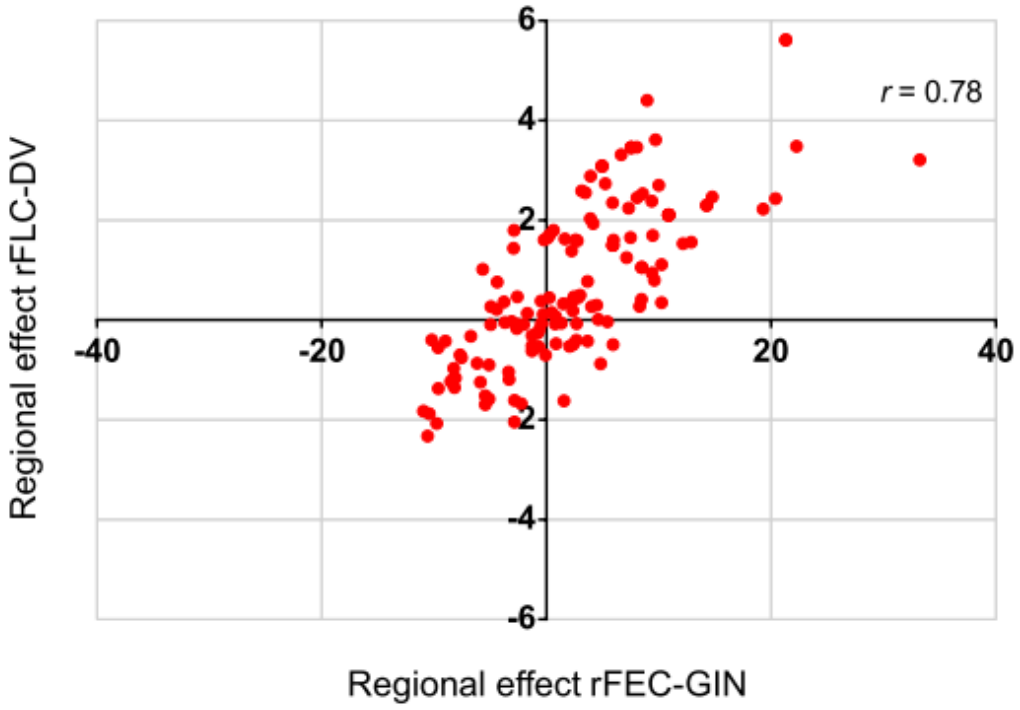

Figure 9. Correlations based on SNP effects between rFLC-DV and rFEC-GIN within the common ROI on BTA 5 (ROI: bp 6,772,101 – 7,683,220; including the *NAV3* gene).

SNP effect correlations between endoparasite traits and test-day traits

The SNP effect correlations between rFEC-GIN, rFEC-FH and rFLC-DV with rMY and rSCS are presented in Figure 10. The correlations between the marker effects for rFEC-GIN and rMY were negative (-0.10 to -0.42) for three ROI. The marker effect correlations between rFEC-GIN and rMY were moderate to large (0.31 to 0.73) for two ROI. Differing correlations between rFEC-GIN and rSCS were estimated for different ROI, i.e., positive correlations (0.02 to 0.32) on BTA 5 and 22 but negative correlations (-0.47 to -0.99) on BTA 4, 18 and 24.

Regarding the ROI identified for rFEC-FH, the correlation between the SNP effects for rFEC-FH and rMY was -0.67, and it was -0.44 between the SNP effects for rFEC-FH and rSCS. The correlations between the SNP effects for rFLC-DV and rMY were in a positive range (0.00 to 0.80) for four ROI, with the largest correlation on BTA 21. Additionally, differing correlations between rFLC-DV and rMY were detected for different ROI from the same BTA on BTA 5. The SNP effect correlations between rFLC-DV and rSCS were positive (0.10 to 0.99) for 12

ROI and neutral or negative (0.00 to -0.15) for two ROI. The correlations between rFLC-DV and rSCS differed for different ROI on BTA 24. Positive correlations for different ROI were observed on BTA 5. For seven ROI on BTA 2, 5 and 26, the correlations between the SNP effects for rFLC-DV and rMY ranged from -0.11 to -0.99, whereas those with rSCS were positive (ranging from 0.11 to 0.99).

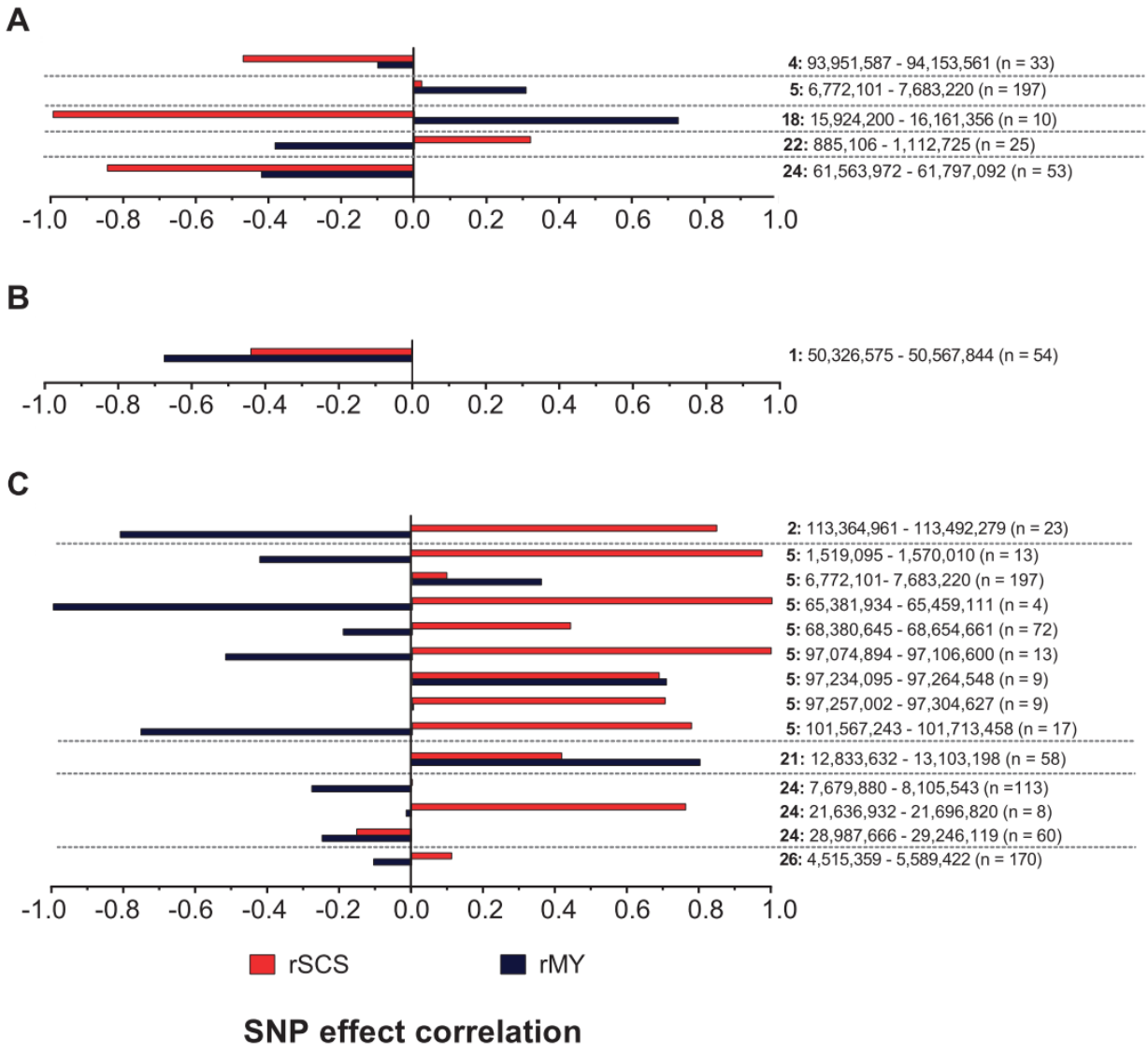

Figure 10. SNP effect correlations between the residuals of endoparasite traits and the residuals of test-day traits somatic cell score (SCS) and milk yield (MY) for the identified genomic regions of potential physiological significance (candidate gene position plus 5 kb up- and downstream) for (A) rFEC-GIN, (B) rFEC-FH and (C) rFLC-DV.

Discussion

Genome-wide association analysis for endoparasite traits

In local breeds with a small population size (e.g., DSN), reduced genetic variation and diversity compared with the intensively selected HF breed is reported (Biedermann et al., 2005; Hartwig et al., 2014). Thus, in comparison with HF or beef cattle breeds (Coppieters et al., 2009; Kim et al., 2014, 2015), other SNP variants associated with endoparasite resistance have been expected. The cattle selection lines best adapted to harsh grassland environments (e.g., New Zealand HF lines, DSN) (McCarthy et al., 2007; Mügge et al., 1999) are often described as more robust and less susceptible to endoparasite infections (May et al., 2017a) One explanation addresses genetic resistance to disease or endoparasite infections via cellular immunological mechanisms and adaptive immune responses, which differ between breeds or selection lines (Bannerman et al., 2008; Stear et al., 2007). Hence, selection signatures were identified when grouping subpopulations according to DSN or HF gene percentages, and when focusing on genomic regions with an impact on disease resistance (Naderi, 2018). In addition, higher levels of genomic homogeneity and of genetic relatedness in DSN than in HF contribute to a decrease in polymorphism, influencing the power to detect marker associations (Riggio et al., 2013).

For rFEC-GIN, the majority of SNP marker associations were detected on BTA 2. Candidate genes were identified on BTA 4, 5, 18, 22 and 24. In contrast, in Angus beef cattle, genomic regions on BTA 3, 5, 15 and 27 were significantly associated ($-\log_{10} P = 4.3$) with GIN infections (Kim et al., 2015). In Scottish Blackface sheep, evidence for associations with GIN infections were detected on OAR 6 and 14 (Riggio et al., 2013), but the SNPs located on OAR 3 and 12 affected FEC in crossbred sheep (Martinique Black Belly x Romane sheep) (Sallé et al., 2012). For Dutch Holstein-Friesian dairy cattle, genome-wide suggestive QTL on BTA 11, 14, 21, 24, 25 and 27 were reported using a within-family analysis based on a dataset of 768 phenotyped cows (Coppieters et al., 2009). In the same study, genome-wide significant QTL were identified on BTA 9 and 19 in an across-family analysis (Coppieters et al., 2009). In agreement with the other studies based on FEC, we also detected significantly associated SNPs for GIN resistance (according to p_{Cand}) on BTA 5, 9 and 24.

The pre-selection of cattle according to phenotypes or allele frequencies, the phenotyping strategy (e.g., utilization of experimental vs. field data) (Kim et al., 2014, 2015), and the differences in trait definitions and parasitological examination techniques are further possible explanations for the different GWAS results in different populations or breeds. In the current study, we did not distinguish among the different species according to the GIN morphotypes.

We assumed that the diversity of GIN species in our study follows the distribution usually reported for cattle, with *Ostertagia ostertagi* as the most common species (Bellet et al., 2016) followed by *Cooperia* spp. and *Trichostrongylus* spp. (Borgsteede et al., 2000). In field data studies, differences in infection exposure among individuals and environments and the variable infection pressure over time explain the reduced power to detect SNP associations and potentially mask the genetic signals (Bishop et al., 2012; Bishop and Woolliams, 2010, 2014). The total number of associations was highest for rFLC-DV although a low prevalence was detected for *D. viviparus* in the multibreed dataset of 1166 cows (May et al., 2017a). False-negative marker associations could be the outcome in a dataset with a low number of infected cows. From this perspective, associations for rFLC-DV should be viewed with caution. However, the low prevalence of *D. viviparus* in our genotyped DSN cows reflected the phenotypic trait distribution in the whole cattle population.

GWAS results for GIN infections in previous studies (Coppieters et al., 2009; Kim et al., 2015) reflect the findings for rFLC-DV. *D. viviparus* and GIN represent the same biological order (Strongylida). Thus, overlaps in marker associations between both species have been expected. We found signals on seven common chromosomes, with impacts on both the rFLC-DV and rFEC-GIN traits. A large number of associations and potential candidate genes were identified on BTA 2 and BTA 24 for rFEC-GIN and rFLC-DV. The *NAV3* gene on BTA 5 was detected for both traits; however, no same SNP was identified to be simultaneously associated. Different SNP variants for different nematode species [trichostrongylids (herein referred to as GIN) and *Nematodirus* spp., which belong to the same biological order] were detected in Scottish Blackface lambs (Riggio et al., 2013). Furthermore, a GWAS for ectoparasites (different tick species) in cattle revealed SNP associations on different chromosomes for the ixodid tick species *A. hebraeum* and *R. evertsi evertsi* (Mapholi et al., 2016).

Regarding FEC-FH, the three significant markers based on p_{Bonf} were not related to potential candidate genes. Efforts to characterize genes or genomic regions for liver fluke traits in ruminants were reported for *Fasciola gigantica* in sheep (Piedrafita et al., 2010). Thus, the current findings present a novelty for enhancing disease resistance to *F. hepatica* in cattle breeds.

Gene annotation and pathway analysis

Our study identified the cytokine-cytokine receptor interaction pathway for the *EGFR* gene for rFEC-GIN. In addition, this pathway was identified in a GWAS for Angus cattle (Kim et al.,

2015). The most interesting finding was the estrogen signaling pathway involving the potential candidate gene *EGFR* for rFEC-GIN. Experiments in mice have indicated that parasites can exploit the hormonal host microenvironment to favor their establishment, growth and reproduction rate (Escobdeo et al., 2005; Romano et al., 2015). In this regard, for the tapeworm *Taenia crassiceps,* an increase in the physiological concentrations of the (host) hormone 17-ß-estradiol was associated with an increase in the reproductive capacity of *T. crassiceps* cysticerci (Escobedo, 2004). Moreover, steroid hormone synthesis (e.g., progesterone, testosterone) influenced the fertility of *Schistosoma mansoni* and increased the length of *Ascaris suum* larvae in its host (Fleming, 1985; Morrison et al., 1985). However, it remains unclear whether similar mechanisms of host exploitation via the regulation of host hormones such as estradiol are also due to infections with GIN. The PI3K-Akt signaling pathway was annotated to several candidate genes for rFEC-GIN. There is evidence that the phosphatidylinositol-3 kinase (PI3K) plays a decisive role in cellular immune response, activated by costimulatory receptors of B and T cells in mice (Fukao and Koyasu, 2003; Koyasu, 2003). Furthermore, the PI3K-Akt signaling pathway was identified for the protozoa *Neospora caninum*, an intracellular parasite that causes high economic losses in the cattle industry (Li et al., 2018).

The activated leukocyte cell adhesion molecule (*ALCAM*) gene on BTA 1 detected for rFEC-FH is related to the cell adhesion molecules (CAMs) pathway, and it plays a crucial role in immune response mechanisms, e.g., cell adhesion interactions of T cells. Interestingly, the same pathway was also detected for *D. viviparus* as being related to the *CDH2* (Cadherin 2, type 1, N-cadherin) gene on BTA 24. Our GWAS revealed several genes and three pathways as being involved in T lymphocyte interactions for rFEC-GIN, rFEC-FH and rFLC-DV. The cellular mechanisms mediated by T lymphocyte recruitment are typical features of immune response to endoparasite (especially helminth) infections in its host (Allen et al., 2011; Hewitson et al., 2009; Maizels and Yazdanbakhsh, 2003). In cattle, natural infections with *F. hepatica* induce Th2-associated reactions, with simultaneous inhibition of Th1 cell activity (Foster and Elsheikka, 2012). Immune response against GIN mainly involves Th2 mechanisms in order to decrease the number of adult worms and of FEC. A mixed Th1/Th2 response follows infections with *D. viviparus* in cattle (Foster and Elsheikka, 2012). An interesting finding was made in Nelore cattle, where the immune response to the GIN species *Cooperia punctata* and *Haemonchus placei* was probably mediated by Th2 cytokines in the resistant cattle group and induced by Th1 cytokines in the susceptible ones (Bricarello et al., 2008; Zaros et al., 2010). Thus, variations in identified immunological pathways might be expected when applying

GWAS based on a stringent case-control (resistance and susceptible groups) design. The *CDH2* and *PCDH15* genes on BTA 24 and 26 for rFLC-DV coded for adhesion molecules, and they were expressed in the cattle selected for either resistance or susceptibility to nematode parasites (Arujo et al., 2009).

Correlations between SNP effects for endoparasite traits

Differing correlations for SNP effects between rFEC-GIN, rFEC-FH and rFLC-DV indicate the complexity of resistance against different infectious agents. In most cases, negative SNP effect correlations were observed between rFEC-FH and rFEC-GIN, implying that genomic selection on improved resistance to *F. hepatica* infections simultaneously increased the susceptibility to GIN. The pedigree-based genetic correlations ranged from -0.10 to 0.17 between different GIN and liver fluke trait definitions (May et al., 2017a; Twomey et al., 2018). One explanation for the negative correlations on a genomic scale between rFEC-FH and rFEC-GIN and for the divergent marker associations as well as gene annotations might be due to the variations in immune response mechanisms for different endoparasite species. In cattle, the specific immune response for *F. hepatica* (antibody response of the IgG1, the cellular response associated with the cytokines interleukin IL4, IL10, TNF-ß) differs from those for GIN and *D. viviparus* (Flynn et al., 2007), where the immune response is predominantly mediated by IgA, IgE, IgG and IgM (Gasbarre et al., 1993; Morris et al., 2003).

The correlations based on the SNP effects between the two nematode traits rFEC-GIN and rFLC-DV were positive for most of the identified ROI, confirming the estimates from a quantitative-genetic study (May et al., 2017a). High positive genetic correlations between the different endoparasite species were reported in sheep (Bishop et al., 2004; Woolaston and Eady, 1995), which simplifies selection strategies.

Correlations between SNP effects for endoparasite and test-day traits

The SNP effect correlations between rFEC-GIN and rMY were negative for three of the five identified ROI, corresponding to the estimates from pedigree-based random regression models (May et al., 2017a). Twomey et al. (2018) detected genetic correlations close to zero between milk yield and antibodies for *Ostertagia ostertagi*, the most common GIN species in cattle. In our study, mostly negative SNP correlations were inferred between rMY and rFLC-DV. Thus, on a genomic scale, breeding for higher milk production reduces larvae shedding of the bovine lungworm. Highly positive SNP effect correlations between rFLC-DV and rMY were detected

for ROI on BTA 5 and 21, indicating a coregulation of both traits in these regions. Another possibility is that the genes affecting FLC for *D. viviparus* and MY were in a low linkage disequilibrium.

Regarding the associations between endoparasite traits and SCS, the SNP effect correlations between rFEC-GIN, rFEC-FH and rFLC-DV with rSCS for the ROI partly reflect quantitative-genetic estimates. May et al. (2017a) estimated positive (i.e., favorable from a breeding perspective) genetic correlations between FEC-GIN and SCS throughout lactation. In contrast, for most of the identified ROI, correlations based on SNP effects were unfavorable between rFEC-GIN and rSCS, but the SNP effect correlations between rFLC-DV and rSCS were positive (i.e., favorable from a breeding perspective). The negative SNP effect correlation between rFEC-FH and rSCS for the ROI on BTA 1 (including the *ALCAM* gene) reflects the pedigree-based estimates, i.e., the negative genetic correlations in the course of lactation (May et al., 2017a). Hence, breeding for reduced FEC for GIN or *F. hepatica* induces an increase in SCS. Vice versa, breeding on low somatic cells implies increasing FEC for GIN and *F. hepatica*. Such findings have practical relevance when developing breeding programs with a focus on both disease resistance and tolerance (König, 2017). In particular, the antagonistic relationship between SCS and egg or larvae counts for endoparasite traits put into question the suitability of SCS as an indicator for udder health. Only moderate phenotypic and genetic correlations between SCS and clinical mastitis, as well as major pathogen susceptibility for cows with extremely low SCS (Martin et al., 2018), are a further justification in this regard. Mechanisms that decrease somatic cells in milk do not necessarily eliminate the causative pathogens during mastitis (Schukken et al., 1991). Phenotypically, the correlations between *F. hepatica* infections and SCS were close to zero (-0.04 to 0.03 for different test-days around the parasitological examination date), reflecting the results from previous studies in HF dairy cow populations (Howell et al., 2015; Mezo et al., 2011). Association analyses between ectoparasite and endoparasite infections with milk composition traits were of great interest in previous studies (Turner et al., 2010; Twomey et al., 2018). Nevertheless, to our knowledge, this is the first approach focusing on the underlying genetic background between endoparasite infections and host defense mechanisms to further pathogen infections (e.g., increase in somatic cells).

Conclusions

The 2-step approach using precorrected phenotype data based on a larger dataset of related genetic lines was a valid approach to estimating SNP marker effects and to inferring possible

candidate genes and biological pathways for endoparasite resistance in a small sample of genotyped dual-purpose DSN cows. Such a methodological approach might be suitable for genomic studies with a focus on novel traits in small populations. In total, 23 potential candidate genes were annotated to SNP marker associations for rFEC-GIN, rFEC-FH and rFLC-DV. A shared ROI (including the *NAV3* gene) was only identified for GIN and *D. viviparus* on BTA 5. Five of the identified possible candidate genes were directly involved in immune response mechanisms. The inferred estrogen signaling pathway is involved in host-parasite interactions, and it appears to be specific for rFEC-GIN. Functional gene annotations identified a common immunological pathway (e.g., cell adhesion molecules pathway for rFEC-FH and rFLC-DV) for different endoparasite traits. The SNP effect correlations between rFEC-GIN and rFLC-DV were quite large for most of the ROI, indicating a partly joint genetic basis for traits representing the same biological order. The negative SNP effect correlation between rSCS and rFEC-FH is in agreements with pedigree-based genetic correlations, and it indicates an antagonistic association between disease resistance for udder and endoparasite infections. Generally, we demonstrated that resistance to the nematodes GIN and *D. viviparus* and to the trematode *F. hepatica* is under polygenic control through a large number of loci with moderate to small effects. The SNP effect correlations for specific endoparasite ROI provided deeper insight into trait associations and contributed to physiological explanations of possible genetic antagonisms between disease resistance and productivity. Predominantly negative SNP effect correlations between GIN or *F. hepatica* with SCS indicate the complexity of immune response mechanisms but also raise critical questions regarding breeding strategies on low somatic cell scores.

References

Ali AKA, Shook GE. 1980. An optimum transformation for somatic cell concentration in milk. Journal of Dairy Science 63:487-490.

Al-Kanaan A. 2016. Heat stress response for physiological traits in dairy and dual-purpose cattle populations on phenotypic and genetic scales. PhD thesis, Faculty of Organic Agriculture, University of Kassel.

Allen JE, Maizels RM. 2011. Diversity and dialogue in immunity to helminths. Nature Reviews Immunology 11:375-388.

Arujo RN, Padilha T, Zarlenga D, Sonstegard T, Connor EE, Van Tassel C, Lima WS, Nascimento E, Gasbarre LC. 2009. Use of a candidate gene array to delineate gene expression patterns in cattle selected for resistance or susceptibility to intestinal nematodes. Veterinary Parasitology 162:106-115.

Bannerman DD, Springer HR, Paape MJ, Kauf ACW, Goff JP. 2008. Evaluation of breed-dependent differences in the innate immune responses of Holstein and Jersey cows to *Staphylococcus aureus* intramammary infection. Journal of Dairy Research 75:291-301.

Barlow R, Piper LR. 1985. Genetic analyses of nematode egg counts in Hereford and crossbred Hereford cattle in the subtropics of New South Wales. Livestock Production Science 12:79-84.

Benavides MV, Sonstegard TS, Kemp S, Mugambi JM, Gibson JP, Baker RL, Hanotte O, Marshall K, Van Tassell C. 2015. Identification of novel loci associated with gastrointestinal parasite resistance in a red Maasai x Dorper backcross population. PLoS One.10:e0122797.

Bellet C, Green MJ, Vickers M, Forbes A, Berrx E, Kaler J. 2016. *Ostertagia spp.*, rumen fluke and liver fluke single- and poly-infections in cattle: An abattoir study of prevalence and production impacts in England and Wales. Preventive Veterinary Medicine 132:98-106.

Biedermann G, Poppinga O, Weitemyer I. 2005. Die genetische Struktur der Population des Schwarzbunten Niederungsrindes. Züchtungskunde 77:3-14.

Bishop SC, Jackson F, Coop RL, Stear MJ. 2004. Genetic parameters for resistance to nematode infections in Texel lambs and their utility in breeding programmes. Animal Science 78:185-194.

Bishop SC, Woolliams JA. 2010. On the genetic interpretation of disease data. Plos One. 5:e8940.

Bishop SC. 2012. A consideration of resistance and tolerance for ruminant nematode infections. Frontiers in Genetics. 3:168.

Bishop SC, Doeschl-Wilson AB, Woolliams JA. 2012. Uses and implications of field disease data for livestock genomic and genetics studies. Frontiers in Genetics 3:114.

Bishop SC, Woolliams JA. 2014. Genomics and disease resistance in livestock. Livestock Science 166:190-198.

Bisset SA, Morris CA, McEwan JC, Vlassoff A. 2001. Breeding sheep in New Zeland that are less reliant on anthelmintics to maintain health and productivity. New Zealand Veterinary Journal 49: 236-246.

Borgsteede FHM, Tibben J, Cornelissen JBWJ, Agneessens J, Gaasenbeek CPH. 2000. Nematode parasites of adult dairy cattle in the Netherlands. Veterinary Parasitology 89:287-296.

Bricarello PA, Zaros LG, Coutinho LL, Rocha RA, Silva MB, Kooyman FN, De Vries E, Yatsuda AP, Amarante AFT. 2008. Immunological responses and cytokine gene expression analysis to *Cooperia punctata* infections in resistant and susceptible Nelore cattle. Veterinary Parasitology 155:95-103.

Browning BLL, Browning SRR. Genotype Imputation with millions of reference samples. 2016;98:116–126. doi:10.1016/j.ajhg.2015.11.020. [accessed 2017 Apr 20]. http://www.sciencedirect.com/science/article/pii/S0002929715004917?via%3Dihub.

Burrow HM. 2001. Variances and covariances between productive and adaptive traits and temperament in a composite breed of tropical beef cattle. Livestock Production Science 70:213-233.

Charlier J, Clearebout E, Duchateau L, Vercruysse J. 2005. A survey to determine relationships between bulk tank milk antibodies against *Ostertagia ostertagi* and milk production parameters. Veterinary Parasitology 129:67-75.

Charlier J, Duchateau L, Clearebout E, Williams D, Vercruysse J. 2007. Associations between anti-*Fasciola hepatica* antibody levels in bulk-tank milk samples and production parameters in dairy herds. Preventive Veterinary Medicine 78:57-66.

Charlier J, De Cat A, Forbes A, Vercruysse J. 2009. Measurement of antibodies to gastrointestinal nematodes and liver fluke in meat juice of beef cattle and associations with carcass parameters. Veterinary Parasitology 166:235-240.

Charlier J, van der Voort M, Kenyon F, Skuce P, Vercruysse J. 2014. Chasing helminths and their economic impact on farmed ruminants. Trends in Parasitology 30:361-367.

Coppieters W, Mes THM, Druet T, Farnir F, Tamma N, Schrooten C, Cornelissen AWCA, Georges M, Ploeger HW. 2009. Mapping QTL influencing gastrointestinal nematode burden in Dutch Holstein-Friesian dairy cattle. BMC Genomics 10:96.

Daetwyler HD, Capitan A, Pausch H, Stothard P, van Binsbergen R, Brøndum RF, et al. 2014. Whole-genome sequencing of 234 bulls facilitates mapping of monogenic and complex traits in cattle. Nature Genetics 46: 858–865.

Davies G, Stear MJ, Benothman M, Abuagob O, Kerr A, Mitchell S, Bishop SM. 2006. Quantitative trait loci associated with parasitic infection in Scottish blackface sheep. Heredity 96:252-258.

Dominik S. 2005. Quantitative trait loci for internal nematode resistance in sheep: a review. Genetics Selection Evolution 37:38-96.

Durinck S, Moreau Y, Kasprzyk A, Davis S, De Moor B, Brazma A, Huber W. 2005. BioMart and Bioconductor: a powerful link between biological databases and microarray data analysis. Bioinformatics 21:3439–3440.

Durinck S, Spellman P, Birney E, Huber W. 2009. Mapping identifiers for the integration of genomic datasets with the R/Bioconductor package biomaRt. Nature Protocols 4:1184–1191.

ENSEMBL genome browser. http://www.ensembl.org/index.html.

Escobedo EG. 2004. Molecular mechanism involved in the differential effects of sex steroids on the reproduction and infectivity of *Taenia crassiceps*. Journal of Parasitology 90:1235-1244.

Escobedo G, Roberts CW, Carrero JC, Morales-Montor J. 2005. Parasite regulation by host hormones: an old mechanism of host exploitation? Trends in Parasitology 21:588-593.

Fleming MW. 1985. *Ascaris suum*; role of ecdysteroids in molting. Experimental Parasitology 60:207-210.

Flynn RJ, Mannion C, Golden O, Hacariz O, Mulcahy G. 2007. Experimental *Fasciola hepatica* infection alters responses to tests used for diagnosis of bovine tuberculosis. Infection and Immunity 75:1373-1381.

Foster N, Elsheikka HM. 2012. The immune response to parasitic helminths of veterinary importance and its potential manipulation for future vaccine control strategies. Parasitology Research 110:1587-1599.

Fukao T, Koyasu S. 2003. PI3K and negative regulation of TLR signaling. Trends in Immunology 24:358-363.

Gasbarre LC, Leighton EA, Davies CJ. 1993. Influence of host genetics upon antibody responses against gastrointestinal nematode infections in cattle. Veterinary Parasitology 46:81-91.

Gasbarre LC, Leighton EA, Sonstegard T. 2001. Role of the bovine immune system and genome in resistance to gastrointestinal nematodes. Veterinary Parasitology 98:51-64.

Gasbarre LC. 2014. Anthelmintic reistance in cattle nematodes in the US. Veterinary Parasitology 204:3-11.

Gordon DK, Roberts LCP, Lean N, Zadoks RN, Sargison ND, Skuce PJ. 2013. Identification of the rumen fluke, *Calicophoron daubneyi*, in GB livestock: possible implications for liver fluke diagnosis. Veterinary Parasitology 195:65-71.

Hartwig S, Bennewitz J. 2014. Züchterische Aspekte zur Konsolidierung und Weiterentwicklung lokaler Rinderpopulationen. Züchtungskunde 86:19-24.

Hayes BJ, Bowman PJ, Chamberlain AC, Verbyla K, Goddard ME. 2009. Accuracy of genomic breeding values in multi-breed dairy cattle populations. Genetics Selection Evolution 41:51-60.

Hewitson JP, Grainger JR, Maizels RM. 2009. Helminth immunoregulation: the role of parasite secreted proteins in modulating host immunity. Molecular and Biochemical Parasitology 167:1-11.

Holzhauer M, van Schaik G, Saatkamp HW, Ploeger HW. 2011. Lungworm outbreaks in adult dairy cows: estimating economic losses and lessons to be learned. Veterinary Record 196:494.

Hou Y, Liu GE, Bickhart DM, Matukumalli LK, Li C, Song J, Gasbarre LC, Van Tassell CP, Sonstegard TS. 2012. Genomic regions showing copy number variation associate with resistance or susceptibility to gastrointestinal nematodes in Angus cattle. Functional & Integrative Genomics 12:81-92.

Howell A, Baylis M, Smith R, Pinchbeck G, Williams D. 2015. Epidemiology and impact of *Fasciola hepatica* exposure in high-yielding dairy herds. Preventive Veterinary Medicine 121:41-48.

Huang DW, Sherman BT, Lempicki RA. 2008. Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. Nature Protocols 4:44–57.

Jaeger M, Brügemann K, König S. 2016. Genetic relationships and trait comparison between and within lines of local dual-purpose cattle. 67th Annual meeting of the European Association for Animal Production, Belfast, Irland.

Kanehisa M, Goto S, Sato Y, Kawashima M, Furumichi M, Tanabe M. 2014. Data, information, knowledge and principle: back to metabolism in KEGG. Nucleic Acids Research 42:199-205.

Karoui S, Carabaño MJ, Díaz C, Legarra A. 2012. Joint genomic evaluation of French dairy cattle breeds using multi-trait models. Genetics Selection Evolution 44:39-49.

Kim ES, Sonstegard TS, Silva MV, Gasbarre LC, van Tassell CP. 2014. Identification of quantitative trait loci affecting gastrointestinal parasite resistance in an experimental Angus population. Animal Genetics 45:117-121.

Kim ES, Sonstegard TS, Silva MV, Gasbarre LC, van Tassell CP. 2015. Genome-wide scan of gastrointestinal nematode resistance in closed Angus population selected for minimized influence of MHC. PLoS One. 10:e0119380.

Knubben-Schweizer G, Deplazes P, Torgerson PR, Rapsch C, Meli ML, Braun U. 2010. Bovine fasciolosis in Switzerland: relevance and control. Schweizer Archiv für Tierheilkunde. 152:223-229.

Korkuć P, Arends D, Scheper C, May K, König S, Brockmann GA. 2017. 1-step versus 2-step imputation: a case study in German Black Pied cattle. Proceedings of the World Congress on Genetics Applied to Livestock Production 11:282.

König S, Simianer H, Swalve HH. 2002. Genetic relationships between dairy performance under large-scale farm and family farm conditions estimated from different groups of common sires. 7th World Congress on Genetics, Montpellier. CD-Rom Communication 18:12.

König S. Genetic background of resistance, tolerance and resilience traits in dairy cattle. 2017. 68th Annual meeting of the European Association for Animal Production, Tallinn, Estonia.

Koyasu S. 2003. The role of PI3K in immune cells. Nature Immunology 4:313-319.

Li S, Gong P, Tai L, Li X, Wang X, Zhao C, Zhang X, Yang Z, Yang J, Li J, Zhang X. 2018. Extracellular vesicles secreted by *Neospora caninum* are recognized by Toll-like receptor 2 and modulate host cell innate immunity through the MAPK signaling pathway. Frontiers in Immunology 9:1633.

Li M-X, Yeung JMY, Cherny SS, Sham PC. 2012. Evaluating the effective numbers of independent tests and significant *p*-value thresholds in commercial genotyping arrays and puclic imputation reference datasets. Human Genetics 131:747–756.

Liu GE, Brown, T, Hebert DA, Cardone MF, Hou Y, Choudhary RK, Schaffer J, Amazu C, Connor EE, Ventura M, Gasbarre LC. 2011. Intitial analysis of copy number variations in cattle selected for reistance or susceptibility to intestinal nematodes. Mammalian Genome 22:111-121.

Martin P, Barkema HW, Brito LF, Narayana SG, Miglior F. 2018. Symposium review: Novel strategies to genetically improve mastitis resistance in dairy cattle. Journal of Dairy Science 101:2724-2736.

May K, Brügemann K, König S, Strube C. 2017. Patent gastrointestinal nematode infections in organically and conventionally pastured dairy cows and their impact on individual milk and fertility parameters. Veterinary Parasitology 245:119-127.

May K, Brügemann K, Yin T, Scheper C, Strube C, König S. 2017. Genetic line comparisons and genetic parameters for endoparasite infections and test-day milk production traits. Journal of Dairy Science 100:7330-7344.

Maizels RM, Yazdanbakhsh M. 2003. Immune regulation by helminth parasites: cellular and molecular mechanisms. Nature Reviews Immunology 3:733-743.

Mapholi NO, Maiwashe A, Matika O, Riggio V, Bishop SC, MacNeil MD, Banga C, Taylor JF, Dzama K. 2016. Genome-wide association study of tick resistance in South African Nguni cattle. Ticks and Tick-borne Diseases 7:487-497.

McCarthy S, Horan B, Dillon P, O'Connor P, Rath M, Shalloo L. 2007. Economic comparison of divergent strains of Holstein-Friesian cows in various pasture-based production systems. Journal of Dairy Science 90:1493-1505.

McMahon C, Bartley DJ, Edgar HWJ, Ellison SE, Barley JP, Malone FE, Hanna REB, Brennan GP, Fairweather I. 2013. Anthelmintic resistance in Northern Ireland (I): Prevalence of resistance in ovine gastrointestinal nematodes, as determined through fecal egg count reduction testing. Veterinary Parasitology 195:122-130.

Mehmood K, Zhang H, Sabir AJ, Abbas RZ, Ijaz M, Durrani AZ, et al. 2017. A review on epidemiology, global prevalence and economical losses of fasciolosis in ruminants. Microbial Pathogenesis 109:253-262.

Mezo M, Gonzalez-Warleta M, Antonio Castro-Hermida J, Muino L, Ubeira FM. 2011. Association between anti-*F. hepatica* antibody levels in milk and production losses in dairy cows. Veterinary Parasitology 180:237-242.

Morris, CA, RS Green, NG Cullen, SM Hickey. 2003. Genetic and phenotypic relationships among faecal egg count, anti-nematode antibody level and live weight in Angus cattle. Animal Science 76:167-174.

Morrison DD, Vandee Waa E, Bennett, JL. 1985. Effects of steroids and steroid synthesis inhibitors on fecundity of *Schistosoma mansoni in vitro*. Journal of Chemical Ecology 12:1901-1908.

Mügge B, Lutz WE, Südbeck H, Zelfel S. 1999. Deutsche Holsteins: Die Geschichte einer Zucht. Verlag Eugen Ulmer.

National Center for Biotchnology Information (NCBI): Bovine genome resource, http://ncbi.nlm.nih.gov.

Naderi S. 2018. Exploring the potential of machine learning methods and selection signature analyses for the estimation of genomic breeding values, the estimation of SNP effects and the identification of possible candidate genes in dairy cattle. PhD thesis, Institute of Animal Breeding and Genetics, Justus-Liebig-University of Gießen.

Oliveira MCS, Alencar MM, Chagas ACS, Giglioti R, Oliveira NH. 2009. Gastrointestinal nematode infection in beef cattle of different genetic groups in Brazil. Veterinary Parasitololy 166:249-254.

Piedrafita D, Spithill TW, Smith RE, Raadsma HW. 2010. Improving animal and human health through understanding liver fluke immunology. Parasite Immunology 32:572-581.

Pimentel ECG, Bauersachs S, Tietze M, Simianer H, Tetens J, Thaller G, Reinhardt F, Wolf E, König S. 2011. Exploration of relationships between production and fertility traits in dairy cattle via association studies of SNPs within candidate genes derived by expression profiling. Animal Genetics 42:251-262.

Purcell S, Neale B, Todd-Brown K, Thomas L, Ferreira MA. 2007. PLINK: a tool set for whole-genome association and population-based linkage abalyses. American Journal of Human Genetics 81:559-575.

Riggio V, Matika O, Pon-Wong R, Stear MJ, Bishop SC. 2013. Genome-wide association and regional heritability mapping to identify loci underlying variation in nematode resistance and body weight in Scottish Blackface lambs. Heredity 110:420-429.

Romano MC, Jiménez P, Miranda-Brito C, Valdez RA. 2015. Parasites and steroid hormones: corticosteroid and sex steroid synthesis, their role in the parasite physiology and development. Frontiers in Neuroscience 9:224.

Sallé G, Jacquiet P, Gruner L, Cortet J, Sauvé C, Prévot F. 2012. A genome scan for QTL affecting resistance to *Haemonchus contortus* in sheep. Journal of Animal Science 90:4690-4750.

SAS Institute Vers. 9.4. SAS Insitute, Inc., Cary, NC.50.

Sayre BL, Harris GC. 2011. Systems genetics approach reveals candidate genes for parasite resistance from quantitative trait loci studies in agricultural species. Animal Genetics 43:190-198.

Schöpke K, Swalve HH. 2016. Review: Opportunities and challanges for small populations of dairy cattle in the era of genomics. Animal 10:1050-1060.

Schukken YH, Grommers FJ, Van de Geer D, Erb HN, Brand A. 1991. Risk factors for clinical mastitis in herds with a low bulk milk somatic cell count. 2. risk factors for *Escherichia coli* and *Staphylococcus aureus*. Journal of Dairy Science 74:826-832.

Stear MJ, Doligalska M, Donskow-Schmelter K. 2007. Alternatives to anthelmintics for the control of nematodes in livestock. Parasitology 134:139-151.

Sutherland IA, Leathwick DM. 2011. Anthelmintic resistance in nematode parasites of cattle: a global issue? Trends in Parasitology 27:176-181.

Turner LB, Harrison BE, Bunch RJ, Porto Neto LR, Li Y, Barendse W. 2010. A genome-wide association study of tick burden and milk composition in cattle. Animal Production Science 50:235-245.

Twomey AJ, Sayers RG, Carroll RI, Byre N, O'Brien E, Doherty ML, McClure JC, Graham DA, Berry DP. 2016. Genetic parameters for both a liver damage phenotype caused by *Fasciola hepatica* and antibody response to *Fasciola hepatica* phenotype in dairy and beef cattle. Journal of Animal Science 94:4109-4119.

Twomey AJ, Carroll RI, Doherty ML, Byrne N, Graham DA, Sayers RG, Blom A, Berry DP. 2018. Genetic correlations between endo-parasite phenotypes and economically important traits in dairy and beef cattle. Journal of Animal Science 96:407-421.

Vercruysse J, Clearebout E. 2001. Treatment vs non-treatment of helminth infections in cattle: defining the threshold. Veterinary Parasitology 98:195-214.

Wall R, Strong L. 1987. Environmental consequences of treating cattle with the antiparasitic drug ivermectin. Nature 327:418-421.

Wellcome Trust Case Control Consortium. 2007. Genome-wide association study of 14,000 cases of seven common diseases and 3,000 shared controls. Nature 447:661–678.

Woolaston RR, Eady SJ. 1995. Australian research on genetic resistance to nematode parasites. In: Gray DG, Woolaston RR, Eaton B, editors. Breeding for resistance to infectious diseases in small ruminants. Australia, Canberra: Australian Centre of International Agricultural Research; p. 1-12.

Xu L, Hou Y, Bickhart DM, Song J, van Tassell CP, Sonstegard TS, George EL. 2014. A genome-wide survey reveals a deletion polymorphism associated with resistance to gastrointestinal nematodes in Angus cattle. Functional & Integrative Genomics 14:333-339.

Yang J, Lee SH, Goddard ME, Visscher PM. 2011. GCTA: a tool for genome-wide complex trait analysis. American Journal of Human Genetics 88:76-82.

Zaros LG, Bricarello PA, Amarante AF, Rocha RA, Kooyman FN, De Vries E, Coutinho LL. 2010. Cytokine gene expression in response to *Haemonchus placei* infections in Nelore cattle. Veterinary Parasitology 171:68-73.

KAPITEL 4

Allele substitution and dominance effects of *CD166/ALCAM* gene polymorphisms for endoparasite resistance and test-day traits in a small cattle population using logistic regression analyses

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Abstract

The study investigated the effects of four single nucleotide polymorphisms (SNPs) in the activated leukocyte cell adhesion molecule (*ALCAM*) gene on liver fluke (*Fasciola hepatica*) infections (FH-INF), gastrointestinal nematode infections (GIN-INF) and disease indicator traits (e.g., somatic cell score [SCS], fat-to-protein ratio [FPR]) in German dual-purpose cattle (DSN). A genome-wide association study inferred the chip SNP *ALCAMc.73+32791A>G* as a candidate for *F. hepatica* resistance in DSN. Because of the crucial function of *ALCAM* in immune responses, SNPs in the gene might influence further resistance and performance traits. Causal mutations were identified in exon 9 (*ALCAMc.1017T>C*) and intron 9 (*ALCAMc.1104+10T>A*, *ALCAMc.1104+85T>C*) in a selective subset of 94 DSN cows. We applied logistic regression analyses for the association between SNP genotypes with residuals for endoparasite traits (rINF-FH, rGIN-INF) and estimated breeding values (EBVs) for test-day traits. The probability of the heterozygous genotype was estimated in dependency of the target trait. Allele substitution effects for rFH-INF were significant for all four loci. The T allele of the SNPs *ALCAMc.1017T>C* and *ALCAMc.1104+85T>C* was the favourable allele when improving resistance against FH-INF. Significant allele substitution for rGIN-INF was only found for the chip SNP *ALCAMc.73+32791A>G*. We identified significant associations between the SNPs with EBVs for milk fat%, protein% and FPR. Dominance effects for the EBVs of test-day traits ranged from 0.00 to 0.47 SD, and were in the direction of improved resistance for rFH-INF. We estimated favourable dominance effects from same genotypes for rFH-INF and FPR, but dominance effects were antagonistic between rFH-INF and SCS.

Introduction

Diseases caused by endoparasite infections hamper production efficiency in cattle (Charlier et al., 2014). Blanco-Penedo et al. (2012) observed substantial detrimental impact on production performance and health due to gastrointestinal nematode (GIN) infections in dairy cows. Infections with the liver fluke, *Fasciola hepatica*, represent a particularly persistent problem in livestock worldwide (Caminade et al., 2015). Mezo et al. (2011) and Schweizer et al. (2005) addressed the substantial economic losses in dairy farms due to fasciolosis, primarily arising from reduced milk production, impaired fertility and liver condemnation. Control of endoparasite infections in livestock generally bases on preventive or curative utilisation of anthelmintics (Vercruysse et al., 2017). However, Beesley et al. (2017) and Walker et al. (2011) identified substantial genetic diversity of *F. hepatica* within cattle herds and individual cows,

causing resistances against anthelmintics. In this regard, sustainable strategies address aspects of breeding and genetics, i.e., the identification of host candidate genes influencing resistance against *F. hepatica* infections (May et al., 2019; Twomey et al., 2019).

Gene expression studies in *Fasciola* infected cattle mainly focussed on Interleukin (IL) and Interferon-γ immune genes (Ingale et al., 2010; Mendes et al., 2013). Zhang et al. (2018) observed an up- and downregulation of several immune genes (e.g., *TLR4, IL-6, CD86*) in the liver of *Fasciola* infected water buffaloes. Recently, May et al. (2019) applied a genome-wide association study (GWAS), and they identified a single nucleotide polymorphism (SNP) *ALCAMc.73+32791A>G* (*rs*110835791) in intron 2 in the activated leukocyte cell adhesion molecule (*ALCAM*) gene on bovine chromosome (BTA) 1. Ongoing gene annotations suggested the *ALCAM* gene as a main candidate regulating immune defence mechanisms with regard to *F. hepatica* infections. The *ALCAM* gene encodes for the activated leukocyte cell adhesion molecule, which refers to the group of adhesion molecules (CAMs). Konno et al. (2001) and Ren et al. (2011) described the crucial role of CAMs in host immune response, e.g. cell adhesion interactions of activated T and B cells during inflammatory infection. Inadequate CAMs expressions impair immune responses, with possible detrimental impact on cow productivity and metabolic health. Differential expression of CAMs (e.g., CD18/CD11, ICAM-1, CD62L/L-selectin) was observed in response to mastitis (Kimura et al., 1999; Oviedo-Boyso et al., 2007), and during the stage of energy deficiency in dairy cattle (Perkins et al., 2001). Hence, we hypothesize that SNPs in the *ALCAM* gene may contribute to variations in resistance indicator traits being linked to pathogen-induced or metabolic disease (e.g., milk somatic cell score [SCS], persistency, fat-to-protein ratio [FPR], respectively). Gengler (1996) addressed the strong associations between disease susceptibility and poor persistency, indicating a substantial lactation curve decline in response to environmental (e.g., pathogen burden) or metabolic stress. The FPR is used as an indicator for energy deficiency and ketosis in dairy cows (Klein et al., 2019), and is considered in selection indices for metabolic disease resistance in Canada (Koeck et al., 2014).

We identified negative SNP effect correlations within *ALCAM* between the mastitis indicator SCS and the trait "faecal egg counts (FEC) of *F. hepatica*" in endangered German dual-purpose cattle (*Deutsches Schwarzbuntes Niederungsrind* [DSN]) (May et al., 2019), a breed being best adapted to harsh environments. The identified relationship between SCS and *F. hepatica* infections (i.e., higher risk for mastitis in liver fluke resistant cows) is particularly interesting for genomic selection strategies to improve overall resistance, since the suppression of Th1

immunity during *F. hepatica* infections increases the susceptibility to bacterial diseases (Brady et al., 1999; Lucena et al., 2017). Such findings suggest deeper genomic insight into *ALCAM* polymorphisms using sequence data, i.e. studying allele substitution and dominance effects for endoparasite resistance and disease indicator traits.

Dominance effects might be important for low heritability functional traits, such as health and fertility in dairy cows (Aliloo et al., 2016; Bolormaa et al., 2015). Aliloo et al. (2016) and Sun et al. (2014) reported improvements in genomic predictions for production and fertility traits when considering both additive and non-additive genetic effects. However, unbiased predictions or estimations of population parameters (e.g. genetic effects, allele substitutions, dominance) ideally require population-wide cow genotyping or sequencing. As an alternative, Henshall and Goddard (1999) suggested a selective genotyping approach combined with logistic regression methodology. Logistic regression analysis was used in selective genotyping studies to identify marker effects in immune genes, e.g. in the bovine lactoferrin gene (Lei et al., 2006), and in the bovine toll-like receptor gene (Bagheri et al., 2013).

The objective of the presented study was a polymorphism screening of exons and flanking intronic regions of the *ALCAM* gene using a selected sample of DSN cows. *ALCAM* gene sequences will be used for the estimation of allele substitution and dominance effects for endoparasite resistance, test-day production traits and indicators for udder diseases and metabolic disorders, considering identified polymorphic loci.

Material and Methods

German Black and White dairy cattle population

The selection of cows based on a sampling design from the "pasture genetics project", which was established in 2007 in Northern Germany. In this project, we focused on genetic line comparisons, quantitative-genetics and genomic mechanisms for endoparasite resistance considering 1,166 German Black and White dairy cows from five selection lines (dataset 1 = DS1). The selection lines included a Holstein Friesian (HF) line selected for milk yield (HFmilk), a HF line suited for grazing conditions (HFpasture), a New Zealand HF line (HFnz), crosses between HF with Jersey, Angler or beef cattle sires (HFcross) and DSN. Parity number of cows ranged from 1 to 11 with a mean of 2.8 lactations. On each farm, faecal samples were collected from all milking cows twice in July and September 2015. Subsequently, we examined repeated measurements per cow for faecal samples for GIN and *F. hepatica* infections. All cows

were exposed to endoparasite infections (access to pasture before 1st of June with > 8 h per day), and not treated with anthelmintics in the sampling year.

Phenotypes

Endoparasite traits were available from all 1,166 cows from DS1, including repeated measurements for 840 cows. The sedimentation technique was used to determine FEC in 10 g faeces per cow for *F. hepatica*. The cows were classified according to their *F. hepatica* infection status (FH-INF) (no egg excretion = non-infected = 0; FEC ≥ 1 = infected = 1), separately for each parasitological examination. Faecal egg counts per gram faeces for GIN were analyzed via modified McMaster technique (Thienpont et al., 1979), with a sensitivity of 25 eggs per gram faeces. Based on the coproscopical examinations, the cows were classified according to their GIN infection status (GIN-INF) (no egg excretion = non-infected = 0; FEC-GIN ≥ 25 = infected = 1), again separately for each parasitological examination. For GIN, the predominant morphotype represented strongylid eggs (Trichostrongylidae [e.g. *Ostertagia ostertagi*] or *Oesophagostomum* and *Bunostomum* spp., respectively), followed by *Strongyloides papillosus* and *Capillaria* spp. eggs (see May et al., 2017).

Test-day production traits for all 1,166 cows considered repeated measurements for milk yield (MY), protein content (Pro%), fat content (Fat%), FPR, milk urea nitrogen (MUN) and somatic cell counts from the whole lactation of the sampling year. Cows with less than five test-day records were excluded from the analysis. The somatic cell counts were log-transformed into somatic cell score: $SCS = \log_2 (SCC/100.000) +3$ (Ali and Shook, 1980). Descriptive statistics for all traits from DS1 are displayed in Table 1.

Table 1. Descriptive statistics of endoparasite traits and test-day production traits from dataset 1.

Endoparasite trait[1]	No. of observations	No. of cows	Mean	SD	Min.	Max.
FH-INF	2006	1166	0.10		0	1.0
GIN-INF	1997	1166	0.28		0	1.0
Production trait	No. of observations	No. of cows	Mean	SD	Min.	Max.
Milk yield (kg)	10,132	1049	22.06	6.98	2.20	57.20
Protein content (%)	10,125	1049	3.37	0.38	2.38	6.32
Fat content (%)	10,125	1049	4.29	0.72	1.75	9.09
Fat-to-protein ratio	10,125	1049	1.28	0.19	0.40	3.29
Milk urea nitrogen (ppm)	10,071	1049	205.54	80.08	50.00	499.00
Somatic cell score	10,115	1049	3.02	1.64	0.01	10.01

[1]FH-INF = Infections status (infected vs. non-infected) for *F. hepatica*; GIN-INF = Infection status (infected vs. non-infected) for gastrointestinal nematodes

Selective genotyping

Imputed genotypes (Illumina HD Bead Chip level, 700k SNP chip) were available for a subset of 148 DSN cows from three farms (Korkuć et al., 2017). Cow genotypes were used in a GWAS, in order to infer genome wide associations with endoparasite resistance (i.e., GIN and *F. hepatica* infections). The selective genotyping approach for the GWAS was explained in detail by May et al. (2019) and considered the three main criteria: Herd prevalences for GIN, extreme GIN phenotypes within herd and minimal pedigree-based genetic relationships among genotyped cows. The SNP *rs*110835791 (*ALCAMc.73+32791A>G* on BTA 1) was the only identified candidate marker for *F. hepatica* resistance (based on the chromosome-wide significance threshold) (Figure 1). Linkage disequilibrium (LD, measured in r^2) was calculated among the SNPs within *ALCAM* and including the 5 kb flanking regions (Hill and Robertson, 1968). The r^2 value between SNP *rs*110835791 and six flanked SNPs ranged from 0.5 to 0.8 (Figure 1). Low linkage ($r^2 \leq 0.5$) was found between SNP *rs*110835791 and all other SNPs.

For ongoing *ALCAM* gene sequencing, we designed a case-control study by selecting 10 DSN cows with extreme phenotypes for *F. hepatica* infections from the DSN farm with the highest

F. hepatica prevalence (dataset 2 = DS2; 5 case individuals = *F. hepatica*-infected cows, egg excretion in both parasitological examinations; 5 control individuals = *F. hepatica* non-infected cows, no egg excretion in both parasitological examinations). The 10 cows were sequenced for all 15 exon regions and the flanking intronic regions in the *ALCAM* gene. Significant differences in allele frequencies between the cases and the controls were identified in exon 9 (results not shown). For validations, we only focussed on sequencing for exon 9 and the flanking intronic regions in the *ALCAM* gene, considering additionally 84 DSN cows. Hence, sequenced genotypes for *ALCAM* exon 9 and intronic flanking regions were available from 94 DSN cows (dataset 3 = DS3). The whole study design and stepwise selection of animals is illustrated in Figure 2.

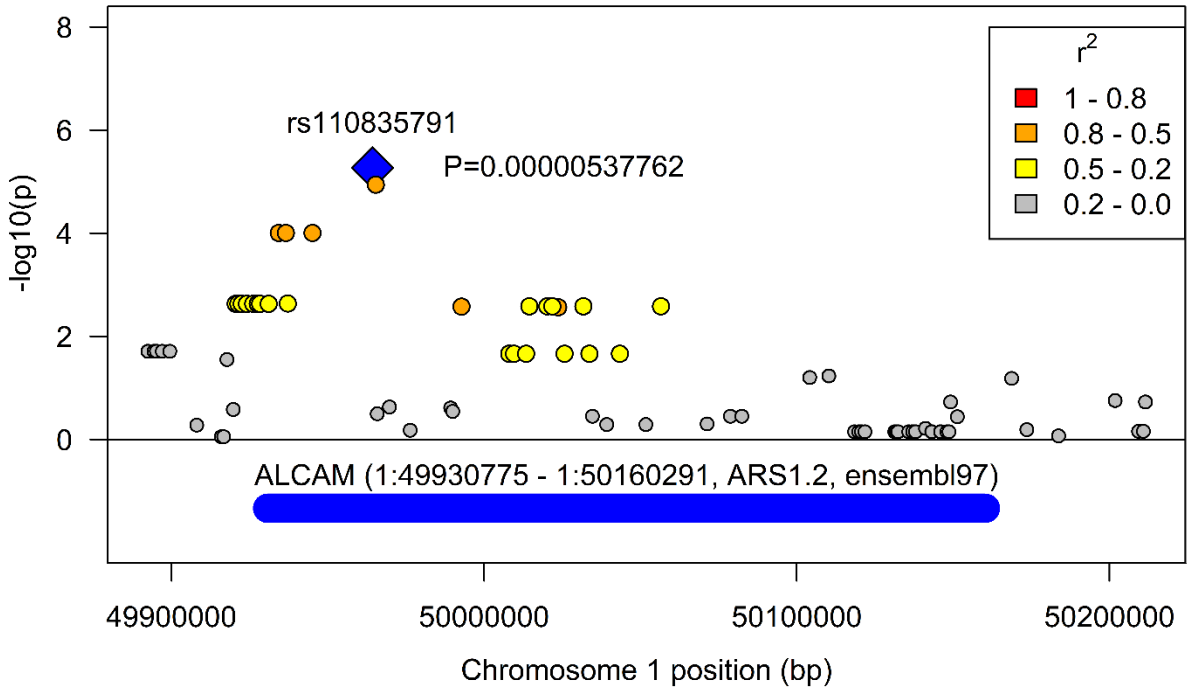

Figure 1. Regional association plot for the activated leukocyte cell adhesion molecule (*ALCAM*) gene on BTA 1. The SNP *rs*110835791 (blue square) was significantly associated (*p*-value was shown) with the trait "Faecal egg counts of *F. hepatica*" in a genome-wide association study (GWAS) in German dual-purpose cattle (DSN). Circles show GWAS *p*-values, with different colours indicating linkage disequilibrium (LD): red: LD 0.8 to 1.0, orange: LD 0.5 to 0.8, yellow: LD 0.2 to 0.5, gray: 0.0 to 0.2.

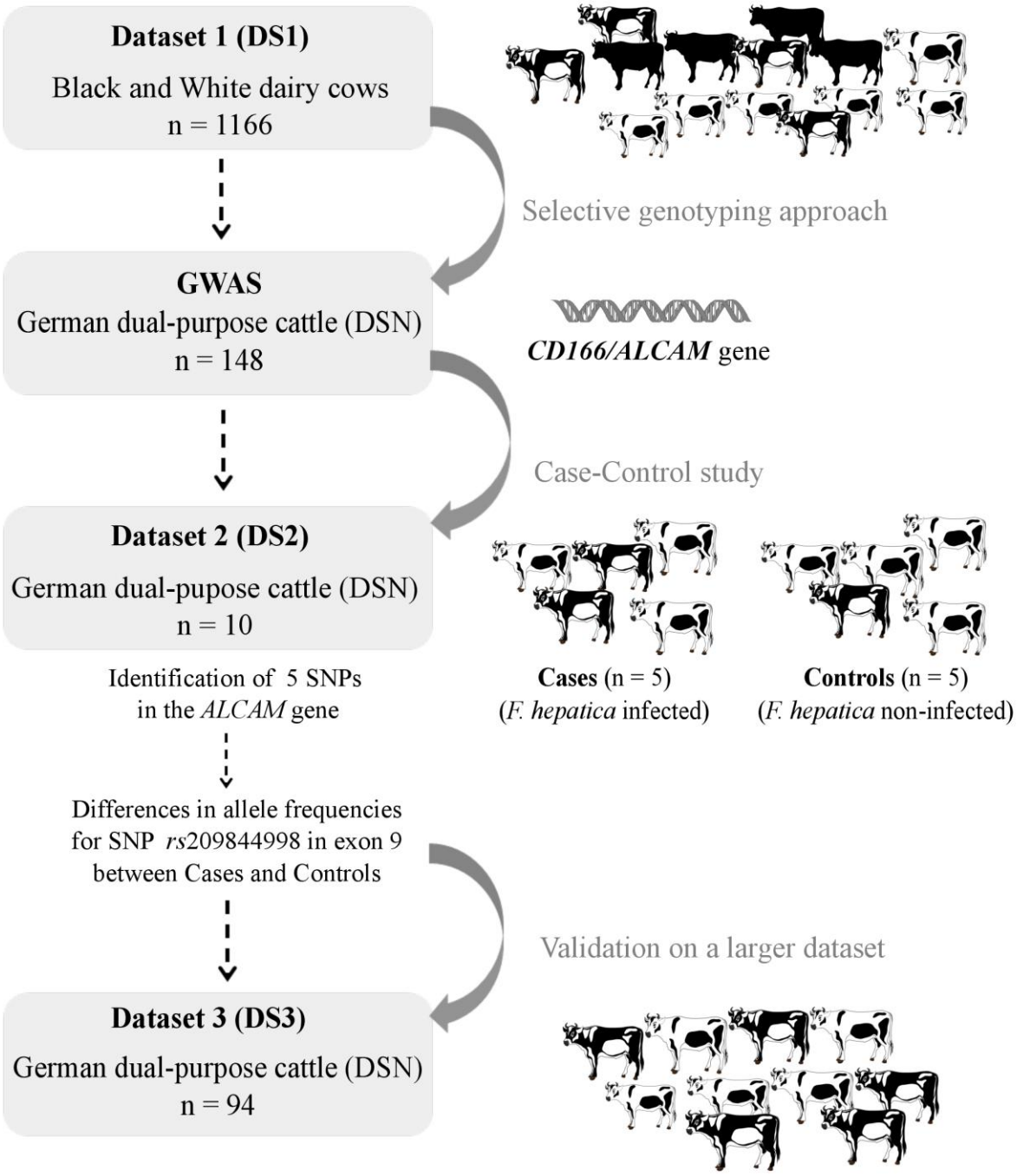

Figure 2. Study design and stepwise selection of cows contributing to the different datasets used in the present study.

Table 3. Details of sequencing primers used for the bovine activated leukocyte cell adhesion molecule gene (*ALCAM*).

Exon	Primer sequences (5'-3')	Product size (bp)	Annealing temperature (°C)
1	Forward: GACTCTGTCGGTGGTCCC	172	62.3
	Reverse: ATAAATCTCCAGGAAGTTTGGGT		
2	Forward: TGTCTCTCTTTTGTTCCTTTCCT	382	55.7
	Reverse: AAACTTCAGGGTGACTCTTTCA		
3	Forward: TGTTGCTCTGACTGTAATACTGA	381	59.5
	Reverse: ACACTACCGAGAGTTCAGCC		
4	Forward: GCAAATATCTGGAATGGGTAGCT	370	59.5
	Reverse: TTTCTGACTGGTGCCTGGAA		
5, 6	Forward: CGTGATGGAACCAAGGTTGTAATA	919	50.0
	Reverse: ATGTTCCAATGTTAAGTCCCAATT		
7	Forward: ACTTGTGTTTGTGACTCTTGCA	299	50.0
	Reverse: CATTCCACAGGTGTCCGTTG		
8	Forward: CAAACTGGTCTCAAAGCTGGC	382	59.5
	Reverse: TAAAAGACCAGCAGAGTTAGACT		
9	Forward: CAAACTGGTCTCAAAGCTGGC	575	53.0
	Reverse: TGGATGCCATAGTGATGTTGAG		
10, 11	Forward: TGGAGAGCTATAATTGTGCCATG	476	53.0
	Reverse: TGCTGTACTTGGTGCAGTGT		
12	Forward: CATTTCCATTTCTAAAGCCATTT	260	59.5
	Reverse: CAGGAAGATGGAATTGAAGATTT		
13, 14	Forward: AAAGGTGACTTTCATATATCAACAAAT	571	59.5
	Reverse: ACATTGAATTTTCTCCCTGTGAA		
15	Forward: CCAAGAAAATAGCCTGTCACTGT	315	59.5
	Reverse: ATCTTTGTAAACCTCACACAGCC		

DNA extraction and sequence analysis

DNA was extracted from blood (200 µl) by using the NucleoSpin®Blood Kit (Macherey Nagel, France) according to the manufacturer's protocol. DNA concentration of all samples was determined using a ND spectrophotometer (NanoDrop Products, USA). For the amplification of each single exon (n = 15) of bovine *ALCAM* for cows from DS2, primers were designed using the software Primer3Plus (Untergasser et al., 2007) based on the reference sequence of *ALCAM* (GenBank: ENSBTAG000000000888), and subsequently synthesized (Microsynth®, Schweiz). A gradient between 50° C and 65° C was used for the PCR to evaluate the optimal annealing temperature for each forward and reverse PCR primer, considering all 15 exons. The PCR reaction was carried out in a volume of 25 µl containing 0.3 µl GoTaq® Flexi DNA Polymerase (5 U/µl) (Promega Corporation, USA), 5 µl 10x buffer, 2,5 µl dNTP mix (2 mM each), 1 µl of each primer (10 µM each), and 1 µl DNA template. Thermocycling conditions were as follows: initial denaturation at 95° C for 4 min, 35 cycles of 95° C for 30 sec, 50° C to 65° C for 30 sec, 72° C for 1 min, and final elongation at 72° C for 1 min. The same protocol was used for sequencing exon 9 and flanking intronic regions considering all cows from DS3. The sequencing primers, corresponding optimal annealing temperatures and product sizes of all sequences are shown in Table 2. The PCR products were subsequently custom sequenced by Sanger sequencing (LGC Genomics Berlin, Germany). Obtained sequences were compared with public reference sequences deposited in the NCBI GenBank, using the Chromas Pro software (version 1.32; http://www.technelysium.com.au/chromas.html).

Statistical analyses

Genotype and allele frequencies

Genotype and allele frequencies were calculated for all SNPs identified in the *ALCAM* gene via sequencing, and for the chip SNP *ALCAMc.73+32791A>G*. Genotype frequencies were tested for deviations from Hardy-Weinberg equilibrium (HWE) by applying a Chi-squared test. The normalized linkage disequilibrium (*D*') and the squared correlation (r^2) as a measure of LD were calculated for all pairwise combinations among the SNPs (Hill and Robertson, 1968). The genotype and allele frequency, HWE and LD calculations were performed using own programming in R version 3.3.4 (R Core Team, 2013). Differences in genotype and allele frequencies between infected and non-infected cows were tested by applying a Chi-squared test, separately for *F. hepatica* and GIN infections. In this regard, cows excreting worm eggs in one

or two consecutive coproscopical examinations (July and/or September 2015) were classified as infected, while non-infected cows excreted no eggs in both coproscopical examinations.

Estimation of SNP marker effects

Precorrection of endoparasite traits

Phenotypes for *F. hepatica* and GIN infection status (FH-INF and GIN-INF, respectively) were precorrected for fixed effects including all 1,166 Black and White cows from DS1. For the precorrection of the binary traits, a generalized linear mixed model with a logit-link function was applied. In this regard, we used the GLIMMIX procedure as implemented in the statistical software SAS version 9.4 (SAS Institute; Cary, NC). The statistical model 1 was:

$$\text{logit}(\pi_{rstuvw}) = \log [\pi_{rstuvw} / (1-\pi_{rstuvw})] = \varphi + F_r + LS_s + P_t + GL_u + SP_v + Cow_w \quad [1]$$

where $\pi_{qrstuvw}$ was the probability of a cow to be infected with *F. hepatica* or GIN; φ was the overall mean effect; F_r was the fixed effect of the r^{th} farm (r = 1,…, 17); LS_s was the fixed effect of the s^{th} lactation stage according to Huth (1995) (s = ≤ 14 days in milk (DIM), 14 - 77 DIM, 78 - 140 DIM, 141 - 231 DIM, ≥232 DIM); P_t was the fixed effect of the t^{th} parity number (t = 1, 2, 3, 4, > 4)*;* GL_u was the fixed effect of the u^{th} genetic line (u = HFmilk, HFpasture, HFnz, DSN, HFcross)*;* SP_v was the fixed effect of the v^{th} parasitological sampling period (v = June/July, September/October)*;* Cow_w was the random cow effect accounting for the two repeated parasitological measurements. Precorrected phenotypes (residuals) for endoparasite traits from model 1 were denoted as rFH-INF and rGIN-INF. Afterwards, residuals were used as pseudo-phenotypes in logistic regression analyses to estimate allele substitution effects.

Estimation of breeding values for test-day traits

A random regression test-day model (RRTDM) with days in milk (DIM) as a time dependent covariate was applied, using the software package DMU (Madsen and Jensen, 2013). Such modelling generates estimated breeding values (EBVs) for test-day production traits (MY, Pro%, Fat%, FPR, MUN, SCS) on a daily basis. The RRTDM 2 was:

$$y_{ijklmno} = HTD_i + P_j + YS_k + \sum_{m=1}^{q} \alpha_{mn} z_m(s) + \sum_{m=1}^{q} \beta_{lm} z_m(s) + \sum_{m=1}^{q} \gamma_{lm} z_m(s) + e_{ijklmno} \quad [2]$$

where $y_{ijklmno}$ was the o^{th} test-day record of the l^{th} cow for MY, Pro%, Fat%, FPR, MUN, SCS; HTD_i was the fixed effect of the i^{th} herd-test-date; $Parity_j$ was the fixed effect of j^{th} parity number (1, 2, 3, 4, >4); YS_k was the fixed effect of k^{th} year-season of last calving (spring,

summer, autumn, winter within each year); q was the the number of covariates (Legendre polynomials of order 2, implying q = 3); α_{mn} was the m^{th} fixed regression coefficient specific to the n^{th} days in milk; β_{lm} was the m^{th} random regression coefficient for the additive genetic effect of cow m by DIM; γ_{lm} was the m^{th} random regression coefficient for the permanent environmental effect of cow m by DIM; z(s) was the vector of covariates of size q (modeled with Legendre polynomials of order 2) describing the shape of the lactation curve of fixed and random regressions evaluated at s DIM; $e_{ijklmno}$ was the random residual effect.

Daily estimated breeding values (EBV_d) for MY (from day 5 to 305 from lactation) were used to calculate a lactation persistency measurement (PERS). According to Pryce et al. (2010), PERS considered the sum of EBV_d from day 39 to 274 in relation to the EBV from day 39 as follows:

$$\sum_{d=39}^{274} EBV_d - 235 \text{ x } EBV_{39}$$

A small value for PERS indicates a high and favourable lactation persistency.

Estimation of allele substitution effects

Allele substitution effects were estimated for i) the chip SNP *ALCAMc.73+32791A>G* in intron 2 and ii) for the three SNPs *ALCAMc.1017T>C*, *ALCAMc.1104+10T>A* and *ALCAMc.1104+85T>C* identified via sequencing in exon 9 and flanking intronic regions (see results 'Sequence analyses and polymorphisms of the *ALCAM* gene').

For the estimation of allele substitution effects at the four different loci, we followed the methodology as introduced by Henshall and Goddard (1999). In the first step, we used a logistic regression model with a logit link function as implemented in the SAS Glimmix macro (Wolfinger and O'Connell, 1993). In this model, *ALCAM* genotypes were considered as dependent variables and coded as binary traits: heterozygous genotypes (e.g. TC) = 1; homozygous genotypes (e.g. TT) = 0. The statistical model for estimating the probability of a heterozygous genotype (e.g. TC) vs. a homozygous genotype (e.g. TT) was defined as follows:

$$\text{logit}(\pi_r) = \log\left[\frac{\pi_r}{1-\pi_r}\right] = a + bY_r \qquad [3]$$

where π_r was the probability of the genotype TC of a cow r; *a* was the intercept; Y_r were the residuals (i.e. the precorrected phenotypes) for endoparasite traits (rFH-INF, rGIN-INF) from model 1 and EBVs for test-day production traits from model 2; *b* was the linear regression of genotype TC on the residuals for endoparasite traits and on the EBVs for test-day production

traits. The significance of linear regression coefficients *b* was tested by the sum of square type 1 tests (Wald-type tests), as implemented in the Glimmix macro.

In the second step, the contrast α of the heterozygous genotype (e.g. TC) to the homozygous genotype (e.g. TT) was estimated using the following equation (Henshall and Goddard, 1999):

$$\alpha = \frac{-1 \pm \sqrt{1 + b^2\sigma_X^2}}{b}$$

with σ_X^2 denoting the variance of the residuals for endoparasite traits or EBVs for test-day production traits in the unselected base population (DS1). Two consecutive runs were applied for each loci and each trait separately to contrasting both homozygous genotypes vs. the heterozygous genotype (for the example above: first contrasting TT to TC, second contrasting CC to TC). Based on α-values from the logistic regression, dominance effects *d* were estimated by contrasting the heterozygous genotype to both homozygous genotypes for endoparasite traits, EBVs for test-day production traits and PERS, considering the three SNPs *ALCAMc.1017T>C*, *ALCAMc.1104+10T>A* and *ALCAMc.1104+85T>C* (Falconer and Mackay, 1996).

Results

Sequence analyses and polymorphisms of the ALCAM gene

In total, five previously described SNPs (ENSEMBL database, http://www.ensembl.org/index.html) were identified in the *ALCAM* gene via sequence analysis of the 10 DSN cows from DS2 (Table 3). One SNP (*ALCAMc.175-46A>G*, *rs*134699600) was detected in intron 2 and another one in intron 7 (*ALCAMc.859-8A>G*, *rs*208667506). As presented in Figure 3, one polymorphism (*ALCAMc.1017T>C*, *rs*209844998) was identified in the coding region in exon 9, flanked by two SNPs in intron 9 (*ALCAMc.1104+10T>A*, *rs*210989424; *ALCAMc.1104+85T>C*, *rs*208125734).

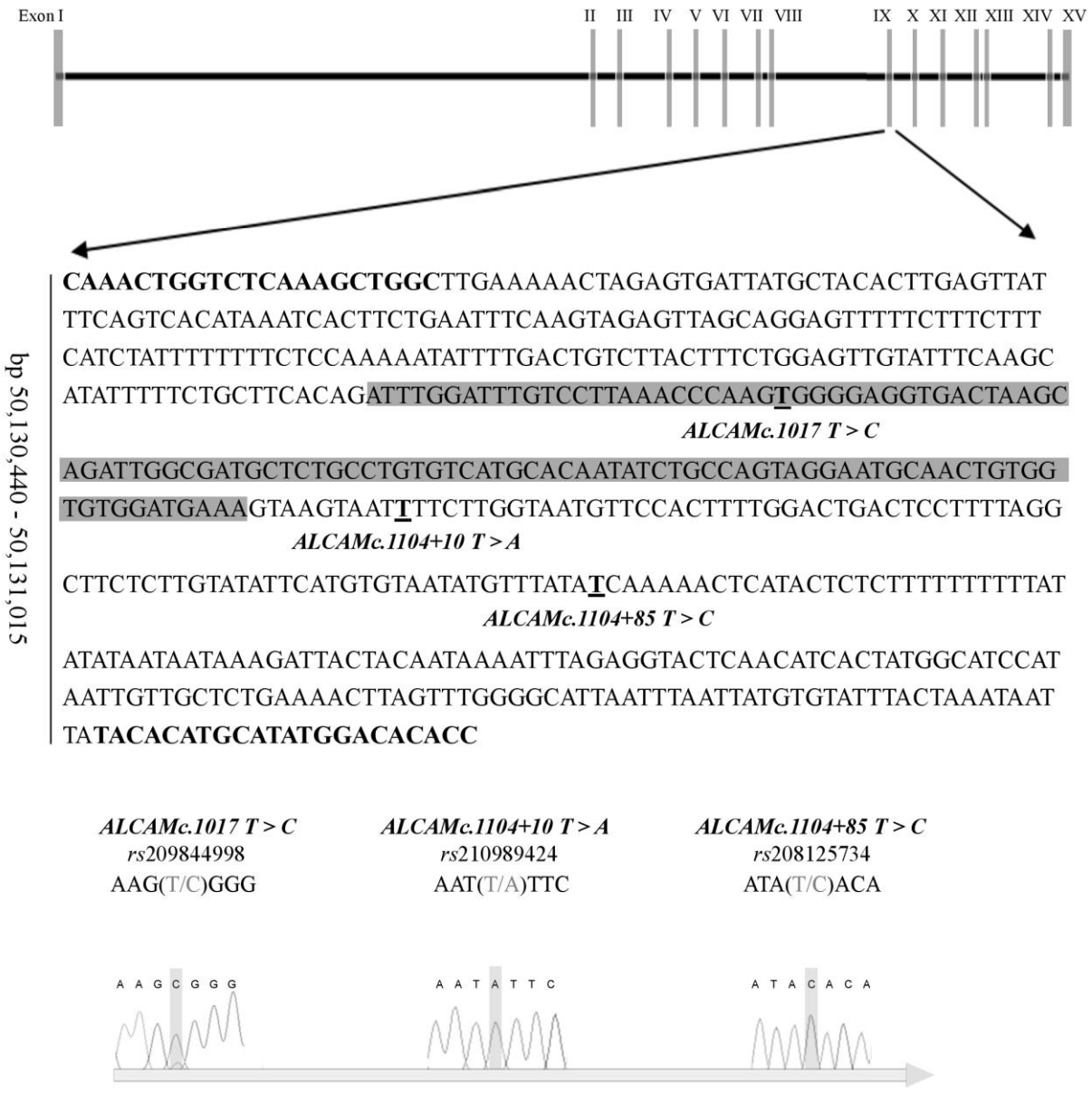

Figure 3. Overview of gene structure (exons are indicated as horizontal grey stripes and labelled by Roman numbers) of the activated leukocyte cell adhesion molecule (*ALCAM*) gene, detailed view of the nucleotide sequence of the coding region exon 9 (with a grey background) and the flanking intronic regions, and blast for example genotypes for single nucleotide polymorphisms *ALCAMc.1017T>C*, *ALCAMc.1104+10T>A* and *ALCAMc.1104+85T>C*. Primers are illustrated in bold. The SNPs *ALCAMc.1017T>C*, *ALCAMc.1104+10T>A* and *ALCAMc.1104+85T>C* are underlined and in bold.

Table 3. Identified SNPs in the bovine *ALCAM* gene on BTA 1 from dataset 2 in German Back Pied cattle (DSN).

SNP	Location	Position	GeneBank accession number
c.175-46A>G	Intron 2	50,095,057	*rs*134699600
c.859-8A>G	Intron 7	50,111,550	*rs*208667506
c.1017T >C	Exon 9	50,130,664	*rs*209844998
c.1104+10T>A	Intron 9	50,130,761	*rs*210989424
c.1104+85T>C	Intron 9	50,130,836	*rs*208125734

Genotype and allele frequencies

The genotype frequencies of the chip SNP *ALCAMc.73+32791A>G* and of the SNPs *ALCAMc.1017T>C, ALCAMc.1104+10T>A* and *ALCAMc.1104+85T>C* from cattle from DS3 are listed in Table 4. For the chip SNP *ALCAMc.73+32791A>G*, the genotype frequency for AA was 2.1%, for AG 17.0%, and for GG 80.9%. For the SNP *ALCAMc.1017T>C,* the genotype frequency for CC was 13.1%, for TC 21.7% and for TT 65.2%. The frequency for allele C was 23.9%, and consequently 76.1% for allele T. Genotype AT for the SNP *ALCAMc.1104+10T>A* had the lowest frequency (18.1%). Genotypes TT and AA were detected with a frequency of 55.3% and 26.6%, respectively. The allele frequency for the A allele was 35.7%, and 64.3% for the T allele. For the SNP *ALCAMc.1104+85T>C,* genotype CC had the lowest frequency (9.1%), followed by TC (22.7%) and TT (68.2%). The T allele was predominant over the C allele (79.5% vs. 20.5%).

The individual frequencies of the genotypes for the three SNPs *ALCAMc.1017T>C*, *ALCAMc.1104+10T>A* and *ALCAMc.1104+85T>C* significantly deviated from HWE ($P \leq 0.05$). For the chip SNP *ALCAMc.73+32791A>G*, the deviation of individual genotype frequencies from HWE was not significant ($P > 0.05$). Significant disequilibrium ($P \leq 0.05$) was present for all pairwise combinations of the three SNPs *ALCAMc.1017T>C*, *ALCAMc.1104+10T>A* and *ALCAMc.1104+85T>C* ($D' > 0.9$). The r^2 values were 0.58 between *ALCAMc.1017T>C* and *ALCAMc.1104+10T>A*, 1.00 between *ALCAMc.1017T>C* and *ALCAMc.1104+85T>C*, and 0.54 between *ALCAMc.1104+10T>A* and *ALCAMc.1104+85T>C* (Figure 4). Moreover, LD was calculated between the chip SNP

ALCAMc.73+32791A>G and the three SNPs located in exon 9 and intron 9. In all cases, the SNPs were in joint equilibrium with r^2 values lower than 0.03 ($P > 0.05$) (Figure 4).

Significant differences between genotype and allele frequencies between *F. hepatica*-infected and non-infected cows from DS3 were detected at all four identified loci (Table 5). The alleles T and G were the predominant alleles for *F. hepatica* non-infected cows. With regard to the GIN infection status, differences in allele frequencies between infected and non-infected cows only were significant for the SNP *ALCAMc.1104+10T>A*.

Allele substitution effects for endoparasite traits

Allele substitution effects for endoparasite traits were estimated for all four loci separately, and ranged from 0.06 to 1.16 SD for rFH-INF and from 0.03 to 0.62 SD for rGIN-INF (Table 6 - 9). Regarding the chip SNP *ALCAMc.73+32791A>G*, the G allele was the predominant allele in the *F. hepatica* non-infected group with significantly lower values for rFH-INF for genotype GG compared to genotype AG ($\alpha = 3.35$; $P = 0.004$) (Table 6). The G allele was the favourable allele for rGIN-INF ($\alpha = 1.00$; $P = 0.009$), albeit genotype frequencies at the chip SNP *ALCAMc.73+32791A>G* were not significant. Additionally, we identified significant allele substitution effects ($P \leq 0.05$) (results not shown) for rFH-INF for three highly linked (LD ≥ 0.5) SNPs downstream from the chip SNP *ALCAMc.73+32791A>G* (Figure 1).

For the SNP *ALCAMc.1017T>C*, allele T was the predominant allele in the *F. hepatica* non-infected group, and accordingly, genotypes TC and CC had higher values for rFH-INF compared to genotype TT (Table 7). Moreover, we found significant lower values for rFH-INF for the genotype TC compared to genotype CC ($\alpha = -3.58$; $P = 0.018$). The GIN infection probability for the heterozygous genotype TC showed intermediate values between both homozygous genotypes TT and CC (Table 7). The difference in rFH-INF between the heterozygous genotype AT compared to the homozygous genotype TT was $\alpha = -1.86$ ($P > 0.05$), and $\alpha = -3.57$ between AT and AA ($P = 0.040$) at SNP *ALCAMc.1104+10T>A* (Table 8). At the same locus, the highest values for rGIN-INF were associated with the genotype TT. For the SNP *ALCAMc.1104+85T>C*, the difference in rFH-INF was significant between genotype TC and genotype CC ($\alpha = -3.81$; $P = 0.021$) (Table 9). For the trait rGIN-INF, the lowest values were found for the genotype CC at the locus *ALCAMc.1104+85T>C* (Table 9).

Table 4. Genotype frequency of the chip SNP *ALCAMc.73+32791A>G*, of the SNP *ALCAMc.1017T>C* in exon 9 and of the SNPs *ALCAMc.1104+10T>A* and *ALCAMc.1104+85T>C* in intron 9 of the *ALCAM* gene for all 94 cows from dataset 3.

SNP	Genotype	Frequency	Percentage
ALCAMc.73+32791A>G	AA	2	2.1
	AG	16	17.0
	GG	76	80.9
	Total	94	
ALCAMc.1017T>C	TT	60	65.2
	TC	20	21.7
	CC	12	13.1
	Total	92	
ALCAMc.1104+10T>A	TT	52	55.3
	AT	17	18.1
	AA	25	26.6
	Total	94	
ALCAMc.1104+85T>C	TT	60	68.2
	TC	20	22.7
	CC	8	9.1
	Total	88	

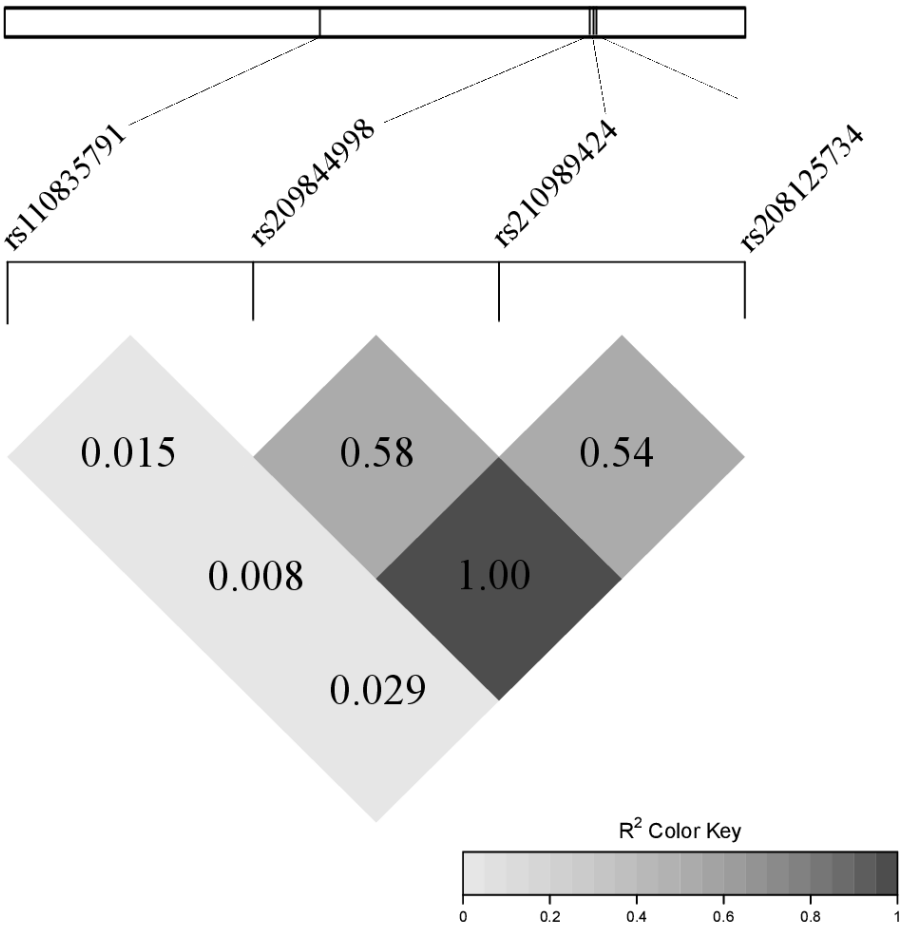

Figure 4. Pairwise linkage disequilibrium in the bovine *ALCAM* gene including the chip SNP *ALCAMc.73+32791A>G* (*rs*110835791) and the three SNPs *ALCAMc.1017T>C*, *ALCAMc.1104+10T>A* and *ALCAMc.1104+85T>C* in the exon 9 and flanking intronic region.

Table 5. Genotype and allele frequencies of the chip SNP *ALCAMc.73+32791A>G*, for the SNP *ALCAMc.1017T>C* in exon 9 and for the SNPs *ALCAMc.1104+10T>A* and *ALCAMc.1104+85T>C* in intron 9 of *ALCAM* in cows from dataset 3 classified by the *Fasciola hepatica* or GIN infection status (infected or non-infected).

ALCAMc.73+32791A>G		Genotype frequency			Allele frequency	
Group	N	AA	AG	GG	A	G
FH infected[1]	17	2	6	9	10	24
FH non-infected[2]	77	0	10	67	10	144
Test-statistic, *P*-value	94	$\chi^2 = 6.376$[3], $P = 0.0116$[3]			$\chi^2 = 15.39$, $P < 0.0001$	
GIN infected[1]	48	0	9	39	9	87
GIN non-infected[2]	46	2	7	37	11	81
Test-statistic, *P*-value	94	$\chi^2 = 0.1290$[3], $P = 0.3591$[3]			$\chi^2 = 0.3293$, $P = 0.5661$	
ALCAMc.1017T>C		Genotype frequency			Allele frequency	
Group		TT	TC	CC	T	C
FH infected	17	8	2	7	18	16
FH non-infected	75	52	18	5	122	28
Test-statistic, *P*-value	92	$\chi^2 = 12.28$, $P = 0.0005$			$\chi^2 = 14.66$, $P = 0.0007$	
GIN infected	47	32	12	3	76	18
GIN non-infected	45	28	8	9	64	26
Test-statistic, *P*-value	92	$\chi^2 = 4.025$, $P = 0.1336$			$\chi^2 = 2.397$, $P = 0.1215$	
ALCAMc.1104+10T>A		Genotype frequency			Allele frequency	
Group		TT	AT	AA	T	A
FH infected	17	7	1	9	15	19
FH non-infected	77	45	16	16	106	48
Test-statistic, *P*-value	94	$\chi^2 = 7.875$, $P = 0.0195$			$\chi^2 = 7.416$, $P = 0.0065$	
GIN infected	48	29	11	8	69	27
GIN non-infected	46	23	6	17	52	40
Test-statistic, *P*-value	94	$\chi^2 = 5.363$, $P = 0.0685$			$\chi^2 = 4.828$, $P = 0.0280$	

ALCAMc.1104+85T>C		Genotype frequency			Allele frequency	
Group		TT	TC	CC	T	C
FH infected	14	8	2	4	18	10
FH non-infected	74	52	18	4	122	26
Test-statistic, *P*-value	88	$\chi^2 = 7.769$, $P = 0.0206$			$\chi^2 = 2.183$, $P = 0.0290$	
GIN infected	46	32	8	6	72	20
GIN non-infected	42	28	12	2	68	16
Test-statistic, *P*-value	88	$\chi^2 = 2.891$, $P = 0.2356$			$\chi^2 = 0.196$, $P = 0.6584$	

[1]*Fasciola hepatica* (FH) infected or GIN infected = positive faecal egg count in one or two consecutive coproscopical examinations; [2]FH non-infected or GIN non-infected = negative faecal egg count in both coproscopical examinations; [3]Calculation of test-statistic and *P*-values is based only on genotypes AG and GG

Table 6. Differences in mean estimated breeding values (EBV) between genotypes of the chip SNP *ALCAMc.73+32791A>G* and regression coefficients (*b*-value) from the logistic model.

Trait	Difference AG vs. GG		
	α (in general units)	α (in SD units)	*b*-value[c]
EBV MY	-0.06	0.13	-0.245
PERS[a]	-2.24	0.01	-0.0001
EBV Fat%	0.01	0.12	1.140
EBV Pro%	0.01	0.19	4.192
EBV FPR	0.001	0.03	1.559
EBV MUN	0.28	0.13	0.059
EBV SCS	0.01	0.08	1.060
rFH-INF[b]	3.35	1.02	0.415**
rGIN-INF[b]	1.00	0.40	0.163**

[a]PERS = A summation of the contribution for each day in the period from day 39 to 274 as a deviation from day 39; [b]rFH-INF, = Residuals for the trait *F. hepatica* infection status (infected vs. non-infected) estimated in a generalized linear mixed model accounting for the fixed effects farm, lactation stage, parity, genetic line and sampling period; [b]rGIN-INF = Residuals for the trait GIN infection status (infected vs. non-infected) estimated in a generalized linear mixed model accounting for the fixed effects farm, lactation stage, parity, genetic line and sampling period. [c]Significance level: *** = $P \leq 0.001$, ** = $P \leq 0.01$, * = $P \leq 0.05$

Table 7. Differences in mean estimated breeding values (EBV) between genotypes of the SNP *ALCAMc.1017T>C* and regression coefficients (*b*-value) from the logistic model.

Trait	Difference TC vs. TT			Difference TC vs. CC		
	α (in general units)	α (in SD units)	*b*-value[c]	α (in general units)	α (in SD units)	*b*-value[c]
EBV MY	-0.03	0.06	-0.108	-0.09	0.17	-0.325
PERS[a]	-8.08	0.04	-0.0002	8.25	0.04	0.0002
EBV Fat%	0.04	0.35	3.374**	0.05	0.50	4.992*
EBV Pro%	0.02	0.38	8.704**	0.02	0.38	8.722
EBV FPR	0.002	0.13	6.904	0.003	0.19	9.963
EBV MUN	0.30	0.14	0.062	0.45	0.21	0.095
EBV SCS	0.008	0.10	1.322	0.003	0.04	0.446
rFH-INF[b]	0.21	0.06	0.02	-3.58	1.09	-0.468*
rGIN-INF[b]	-0.08	0.03	-0.013	1.26	0.50	0.210

[a]PERS = A summation of the contribution for each day in the period from day 39 to 274 as a deviation from day 39; [b]rFH-INF, = Residuals for the trait *F. hepatica* infection status (infected vs. non-infected) estimated in a generalized linear mixed model accounting for the fixed effects farm, lactation stage, parity, genetic line and sampling period; [b]rGIN-INF = Residuals for the trait GIN infection status (infected vs. non-infected) estimated in a generalized linear mixed model accounting for the fixed effects farm, lactation stage, parity, genetic line and sampling period. [c]Significance level: *** = $P \leq 0.001$, ** = $P \leq 0.01$, * = $P \leq 0.05$

Table 8. Differences in mean estimated breeding values (EBV) between genotypes of the SNP *ALCAMc.1104+10T>A* and regression coefficients (*b*-value) from the logistic model.

Trait	Difference AT vs. TT			Difference AT vs. AA		
	α (in general units)	α (in SD units)	*b*-value[c]	α (in general units)	α (in SD units)	*b*-value[c]
EBV MY	-0.07	0.14	-0.281	-0.01	0.02	-0.044
PERS[a]	-15.69	0.07	-0.0004	-14.79	0.06	-0.0003
EBV Fat%	0.05	0.46	4.480**	0.04	0.36	3.431*
EBV Pro%	0.02	0.51	12.07**	0.01	0.17	3.870
EBV FPR	0.004	0.22	11.841*	0.003	0.17	9.207
EBV MUN	0.33	0.15	0.069	0.44	0.20	0.092
EBV SCS	0.01	0.13	1.680	-0.004	0.06	-0.716
rFH-INF[b]	-1.86	0.56	-0.186	-3.57	1.08	-0.465*
rGIN-INF[b]	-0.16	0.06	-0.025	0.55	0.22	0.087

[a]PERS = A summation of the contribution for each day in the period from day 39 to 274 as a deviation from day 39; [b]rFH-INF, = Residuals for the trait *F. hepatica* infection status (infected vs. non-infected) estimated in a generalized linear mixed model accounting for the fixed effects farm, lactation stage, parity, genetic line and sampling period; [b]rGIN-INF = Residuals for the trait GIN infection status (infected vs. non-infected) estimated in a generalized linear mixed model accounting for the fixed effects farm, lactation stage, parity, genetic line and sampling period. [c]Significance level: *** = $P \leq 0.001$, ** = $P \leq 0.01$, * = $P \leq 0.05$

Table 9. Differences in mean estimated breeding values (EBV) of test-day traits between genotypes of the SNP *ALCAMc.1104+85T>C* and regression coefficients (*b*-value) from the logistic model.

Trait	Difference TC vs. TT			Difference TC vs. CC		
	α (in general units)	α (in SD units)	*b*-value	α (in general units)	α (in SD units)	*b*-value
EBV MY	-0.03	0.06	-0.108	-0.07	0.13	-0.250
PERS[a]	-8.08	0.04	-0.0002	-2.24	0.01	-0.0001
EBV Fat%	0.04	0.35	3.374**	0.06	0.52	5.23
EBV Pro%	0.02	0.38	8.704**	0.01	0.31	7.121
EBV FPR	0.002	0.13	6.904	0.002	0.12	6.470
EBV MUN	0.30	0.14	0.062	0.55	0.25	0.115
EBV SCS	0.008	0.10	1.322	0.01	0.12	1.579
rFH-INF[b]	0.21	0.06	0.02	-3.81	1.16	-0.526*
rGIN-INF[b]	-0.08	0.03	-0.013	1.58	0.62	0.272

[a]PERS2 = A summation of the contribution for each day in the period from day 39 to 274 as a deviation from day 39; [b]rFH-INF, = Residuals for the trait *F. hepatica* infection status (infected vs. non-infected) estimated in a generalized linear mixed model accounting for the fixed effects farm, lactation stage, parity, genetic line and sampling period; [b]rGIN-INF = Residuals for the trait GIN infection status (infected vs. non-infected) estimated in a generalized linear mixed model accounting for the fixed effects farm, lactation stage, parity, genetic line and sampling period. [c]Significance level: *** = $P \leq 0.001$, ** = $P \leq 0.01$, * = $P \leq 0.05$

Dominance effects for endoparasite traits

Dominance effects for the trait rFH-INF were in the direction of favourable resistance, as indicated by the negative sign for all SNPs (Table 10). In the case of SNP *ALCAMc.1104+10T>A*, the heterozygous genotype AT was associated with a decrease for rFH-INF, compared to both homozygous genotypes TT and AA. Hence, a value of d = -2.72 representing overdominance, was estimated for the SNP *ALCAMc.1104+10T>A.* Dominance of value d = -1.69 was found for genotype TC of the SNP *ALCAMc.1017T>C,* and of d = -1.80 for genotype TC of the SNP *ALCAMc.1104+85T>C.* Estimates of dominance effects for the trait rGIN-INF were positive in all cases (range of d: 0.20 to 0.75), and thus, not in the direction of desired resistance (Table 9). For the three SNPs *ALCAMc.1017T>C, ALCAMc.1104+10T>A* and *ALCAMc.1104+85T>C,* intermediate values for the heterozygous genotypes were observed.

Table 10. Estimates of dominance effects for the EBVs in test-day production traits and for endoparasite traits for the SNPs *ALCAMc.1017T>C*, *ALCAMc.1104+10 T>A* and *ALCAMc.1104+85T>C*. Values in brackets are dominance effects expressed in SD units.

Trait	SNP in *ALCAM*		
	c.1017 T>C	*c.1104+10 T>A*	*c.1104+85 T>C*
EBV MY	-0.06 (0.12)	-0.04 (0.08)	-0.05 (0.10)
PERS[a]	0.09 (0.00)	-15.24 (0.07)	-5.16 (0.02)
EBV Fat%	0.05 (0.47)	0.05 (0.47)	0.05 (0.47)
EBV Pro%	0.02 (0.44)	0.02 (0.44)	0.02 (0.44)
EBV FPR	0.003 (0.16)	0.01 (0.32)	0.002 (0.11)
EBV MUN	0.38 (0.17)	0.39 (0.18)	0.43 (0.20)
EBV SCS	0.01 (0.08)	0.003 (0.04)	0.01 (0.13)
rFH-INF[b]	-1.69 (0.51)	-2.72 (0.82)	-1.80 (0.55)
rGIN-INF[b]	0.59 (0.23)	0.20 (0.08)	0.75 (0.30)

[a]PERS = A summation of the contribution for each day in the period from day 39 to 274 as a deviation from day 39; [b]rFH-INF, = Residuals for *F. hepatica* infection status; [b]rGIN-INF = Residuals for GIN infection status

Allele substitution effects for test-day traits

Differences in EBVs for test-day traits and for PERS between the heterozygous and both homozygous genotypes are presented in Tables 5 - 8. Allele substitution effects for the EBVs in Fat% and Pro% ranged from 0.12 to 0.52 SD, and for EBVs in FPR from 0.03 to 0.22 SD. Significances for regression coefficients were observed for the EBV in Fat% when contrasting genotype TC to genotype TT at SNP *ALCAMc.1017T>C* (α = 0.04; P = 0.007) and at SNP *ALCAMc.1104+85T>C* (α = 0.04; P = 0.007). Moreover, we found significant differences in EBVs for Fat% for genotype TC vs. genotype CC at SNP *ALCAMc.1017T>C* (α = 0.05; P = 0.022), for AT vs. TT (α = 0.05; P = 0.003) and for AT vs. AA (α = 0.04; P = 0.049) at SNP *ALCAMc.1104+10T>A*. In addition, contrasts for FPR were significant, i.e., for genotype AT vs. TT at SNP *ALCAMc.1104+10T>A* (α = 0.004; P = 0.052). For the EBVs in Pro%, regression coefficients were significant for the SNPs *ALCAMc.1017T*>C (α = 0.02; P = 0.010), *ALCAMc.1104+10T>A* (α = 0.02; P = 0.009) and *ALCAMc.1104+85T>C* (α = 0.02; P = 0.010). We identified low to moderate (range: 0.13 to 0.25 SD) allele substitution effects for the EBV in MUN. Estimates for MUN EBVs were larger for the heterozygous genotype, compared to both homozygous genotypes at the SNPs *ALCAMc.1017T>C*, *ALCAMc.1104+10T>A* and *ALCAMc.1104+85T>C*. The same trend was observed for the EBV in SCS, with higher values for the heterozygous genotypes compared to the homozygous genotypes, except for the comparison of genotype AT vs. AA at the SNP *ALCAMc.1104+10T>A.* Allele substitution effects for SCS EBVs were quite small for all four loci (range: 0.04 to 0.13 SD). For the EBVs in MY and PERS, allele substitution effects ranged from 0.01 to 0.17 SD. Lower values for EBV in MY (unfavourable from a breeding perspective) and PERS (favourable from a breeding perspective) were found for the heterozygous genotypes TC and AT vs. the homozygous genotypes at all four SNPs, expect for genotype TC vs. CC for PERS at the SNP *ALCAMc.1017T>C*.

Dominance effects for test-day traits

Estimates for dominance effect were small (range of d: -0.06 to 0.05) for the EBVs of the test-day production traits MY, Fat%, Pro% and FPR (Table 9). With regard to all three loci, also dominance effects for the EBVs in SCS were close to zero (range of d: 0.003 to 0.01) (Table 9). For PERS, negative values for dominance are favourable, i.e. indicating a higher persistency of the heterozygous genotype compared to the expectation value (= average between both homozygous genotypes). This was the case for the SNPs *ALCAMc.1104+10T>A* and

ALCAMc.1104+85T>C in intron 9, but not for the SNP *ALCAMc.1017T>C* in exon 9. For example, for *ALCAMc.1104+10T>A*, the difference in PERS between genotype TT and genotype AA was -0.9, and the expected value was -0.45. For the MUN EBV, dominance effects were positive for all three SNPs in the range from 0.38 to 0.43 (Table 9).

Discussion

The role of ALCAM in response to disease

This is the first study reporting significant associations between polymorphisms in the *ALCAM* gene and resistance to liver fluke and GIN infections. The *ALCAM* gene encodes for the activated cell adhesion molecule (ALCAM/CD166). This molecule is a member of the immunoglobulin superfamily and acts as a ligand for CD6, a signalling receptor on T and B cells (Swart, 2002). The CD6/ALCAM interaction plays a key role in mediating the binding of T cells to activated leukocytes, and thus, *ALCAM* is of considerable importance in immune response mechanisms during disease (Zheng et al., 2016). In humans, SNPs in the *ALCAM* gene were associated with cancer and autoimmune disease (e.g., rheumatoid arthritis) (King et al., 2004; Wagner et al., 2014; Zhou et al., 2011). Tao et al. (2004) considered the *ALCAM* gene for the construction of a bovine immune-endocrine cDNA microarray and found an increased expression of the gene in bovine mononuclear cells. However, gene expressions in experimentally *F. hepatica* infected sheep were unrelated with up- or down-regulations of the *ALCAM* gene (Alvarez-Rojas et al., 2015; Ruiz-Campillo et al., 2017). Piedrafita et al. (2004) observed variations in immune responses to *F. hepatica* infections between cattle and sheep. Moreover, cattle are more resistant to *F. hepatica* infections compared with sheep because of substantial liver fibrosis and due to the ability to overcome infections by "self-cure" (Hillyer et al., 1996). Hence, *ALCAM* expression variations in the liver tissue due to liver damage and fibrosis might differ between both species. Available gene expression studies in *F. hepatica* infected cattle focussed on genes that are up- or down-regulated during infection (Ingale et al., 2010; Mendes et al., 2013). Ruiz-Campillo et al. (2017) identified an increased expression of the vascular cell adhesion protein 1 (VCAM-1) in inflammatory liver cells of *F. hepatica* infected sheep. Similar to ALCAM, VCAM-1 mediates leukocyte-endothelial cell adhesion, a crucial immune function in the host during parasite infections. Alvarez-Rojas et al. (2015) detected several key metalloproteinases via transcriptomic studies in *F. hepatica* infected sheep. Metalloproteinases are enzymes expressed in the liver in response to hepatic damage (Han et al., 2006). Studies in humans reported strong interactions between metalloproteinase and

ALCAM networks (Lunter et al., 2005; Weidle et al., 2010). Our results suggest analyses of gene expression profiles of *F. hepatica* infected and non-infected cattle, in order to validate the role of the *ALCAM* gene in host-liver fluke interactions in other cattle breeds.

Allele substitution effects

Estimations of allele substitution effects in immune genes for *F. hepatica* and GIN resistance are restricted to sheep and goats. The substitution of an allele at the *DRB1* locus of the major histocompatiblity complex (MHC) influenced GIN resistance in sheep (Charon et al., 2002; Schwaiger et al., 1995). Benavides et al. (2009) reported a significant GIN FEC decrease due to allele substitutions in the *IL-4* gene in a population of Corriedale sheep. However, the effect was not confirmed in Polwarth sheep, indicating breed specific genomic mechanisms. Recently, Alam et al. (2019) identified 10 novel variants in six genes, affecting immune response to the trichostrongylid *Haemonchus contortus* in goats. In our study, we did not distinguish among different GIN species, albeit immune response and pathogenicity can vary between species (Foster and Elsheikka, 2012). We supposed that *Ostertagia ostertagi* and *Cooperia* spp. were the prevalent GIN species as usually reported for cattle (Borgsteede et al., 2000). Nevertheless, resistance to one GIN species might be associated with a lower susceptibility to another GIN species (Gray 1997). *Ostertagia ostertagi* follows a seasonal pattern with the highest peak for worm counts between June and August (Agneessens et al., 2000), while the prevalence for *F. hepatica* infections is the highest in autumn (Kuerpick et al., 2012). Against this background, we conducted the first sampling in July to ensure that the prepatent period for *F. hepatica* and GIN was exceeded after turnout, and the second sampling in September due to the expected high *F. hepatica* prevalence.

In our study, we identified useful marker genotypes for resistance against *F. hepatica* for all four SNPs from the *ALCAM* gene. The substitution with allele G of the chip SNP *ALCAMc.73+32791A>G* decreased the probability for *F. hepatica* infections. Allele T was the predominant and favourable allele for the SNPs *ALCAMc.1017T>C* and *ALCAMc.1104+85T>C*, regarding improvements in liver fluke resistance. Accordingly, possibly due to natural selection and adaptive mechanisms to harsh grassland environments, the allele frequency for the T allele was significantly higher in *F. hepatica* non-infected cows. In contrast to the favourable influence of allele T on *F. hepatica* resistance, the same allele T of the SNPs *ALCAMc.1017T>C* and *ALCAMc.1104+85T>C* increased the probability for GIN infections. The antagonistic effect between GIN and *F. hepatica* for the specific loci is in

agreement with the negative SNP effect correlations considering selected chromosome segments (May et al., 2019). Hence, the selective genotyping approach combined with logistic regressions on probabilities for cow genotypes was a proper method for small samples of sequenced cows and low prevalence traits. From a genetic-statistical perspective and trait definitions, also Tilquin et al. (2003) favoured logistic regressions, because such modelling is independent from assumptions for trait distributions.

We identified significant allele substitution effects for the EBVs of the milk production traits Fat% and Pro% at the three SNPs in exon 9 and flanking intronic regions of the *ALCAM* gene, and for FPR at SNP *ALCAMc.1104+10T>A*. Regarding production trait allele substitution effects, Dusza et al. (2018) identified an influence of a mutation in the *L-selectin* gene on milk protein and on milk fat content. Czarnik et al. (2007) studied a silent point mutation in the *ITBG2* gene encoding the CD18 subunit, and they identified an influence on milk protein content. In the present study, EBVs for Fat% and Pro% were highest for the heterozygous genotype TC of the SNPs *ALCAMc.1017T>C* and *ALCAMc.1104+85T>C*, and for AT of the SNP *ALCAMc.1104+10T>A*. Selection on the T allele (i.e., the favourable allele for *F. hepatica* resistance at the SNPs *ALCAMc.1017T>C* and *ALCAMc.1104+85T>C*) was not significantly associated with genetic improvements for Fat% and Pro%. For the SNP *ALCAMc.1104+10T>A* in intron 9, favourable allele substitution effects were identified for both trait categories disease resistance (FH-INF) and for test-day traits reflecting milk or fat content (Fat%, Pro%, FPR, PERS). Hence, we recommend adding the identified sequence variants on a breed specific SNP chip array, offering breeding potential for simultaneous health and productivity improvements in the DSN breed. Such a specifically designed SNP chip is very important when considering future DSN breeding goals, since *F. hepatica* infections lead to reduced feed intake and a decrease of serum glucose levels in ruminants (Phiri et al., 2007; Kozat and Denizhan, 2010). In causality, low serum glucose levels contribute to negative energy balance, inducing the occurrence of metabolic diseases (e.g. ketosis).

Nevertheless, selection on the T allele or on genotype AT of the SNP *ALCAMc.1104+10T>A* negatively influenced MY, SCS and MUN. The G allele from the chip SNP *ALCAMc.73+32791A>G* favourably influenced the EBVs of the test-day traits MY, MUN and SCS, as well as endoparasite resistance traits (rFH-INF, rGIN-INF). Genotype TC of the SNPs *ALCAMc.1017T>C* and *ALCAMc.1104+85T>C* contributed to a significant decrease of *F. hepatica* infections, but simultaneously to a decrease in MY and an increase in SCS. Hence, we observed an antagonistic association between resistance against endoparasites (FH-INF) and

udder infections (indicator trait SCS), which is in agreement with the antagonistic SNP effect correlations between SCS and FEC for *F. hepatica* from chromosome segments including the *ALCAM* gene (May et al., 2019). Antagonistic associations might be due to a negative genetic relationship between cellular mediated and antibody mediated immune response (Thompson-Crispi et al., 2012; Heriazon et al., 2013). Therefore, genetic selection of cows with increased resistance to helminth infections (e.g., *F. hepatica*) might be associated with increasing susceptibility to bacterial infections (Lucena et al., 2017). Moreover, negative associations between helminth infections and subclinical mastitis were found in sheep on a phenotypic scale (Kordalis et al., 2019).

Generally, allele substitution effects were quite small for the EBVs of the production traits MY and PERS at all four loci. An explanation might be that the identified SNPs in the *ALCAM* gene are not linked to other genes with significant effects on milk volume. Gengler (1996) reported strong relationships between disease susceptibility and persistency in dairy cattle. Thus, lactation persistency was taken into consideration in this study as a trait indicating overall cow robustness (Calus et al., 2013). For the SNP *ALCAMc.1104+10T>A*, same favourable allele substitution effects were identified for PERS and for rFH-INF. Rajala-Schultz et al. (2001) and Roy et al. (2011) suggested MUN as a biomarker for fertility and energy efficiency in lactating cows. In the present study, allele substitution effects for MUN were in a moderate range (0.13 to 0.25 SD), with highest MUN values for the heterozygous genotype. Thus, consequent selection on DSN cows with heterozygous genotypes for the four loci implies a positive genetic trend for MUN, with detrimental impact on female fertility traits (König et al., 2008).

Dominance effects

All dominance effects for rFH-INF were in the direction of improved resistance (i.e., negative values for rFH-INF), indicating that the estimates for the heterozygous genotype were lower than the expectation value (i.e., the average value from both homozygous genotypes). Interestingly, the opposite effect was observed for the trait rGIN-INF (i.e., positive dominance effects for all three loci), with considerably lower *d* values. According to Hill et al. (2008), we expected only small dominance effects (d = -0.06 to 0.05) for production traits with moderate heritabilities, as shown in the present study for the EBVs of MY, Fat%, Pro% and FPR. McClure et al. (2014) and Twomey at al. (2018, 2019) estimated low heritabilities in a range of 0.01 to 0.15 for *F. hepatica* resistance traits (e.g., presence of flukes in the liver, antibody titre) in a population of Irish beef and dairy cattle. Additionally, low heritabilities between 0.07 and

0.21 were reported for GIN resistance traits (FEC, antibody titre) in adult cattle (Coppieters et al., 2009; Twomey et al., 2018). Thus, large dominance effects for rFH-INF (d = -2.72 to -1.69) and rGIN-INF (d = 0.20 to 0.75) reflect the small additive genetic variances and heritabilities for endoparasite resistance traits. However, effects of dominance vary between populations, and might be influenced due to genotype by environment interactions (Georges et al., 2019). Consequently, high values of dominance for endoparasite traits at the *ALCAM* loci in our DSN population may not replicate in other cattle breeds. In the present study, we focused on dominance effects considering the small cattle breed DSN, whereas prior studies addressing parasite resistance in ruminants investigated the effect of favourable alleles in crossbreeding designs (Hanotte et al., 2002; Marshall et al., 2013). Marshall et al. (2013) estimated dominance effects for quantitative trait loci associated with resistance to the gastrointestinal nematode *Haemonchus contortus* in African Red Masaai and Dorper sheep. They reported dominance effects of 0.12 SD for the trait eggs per worm and 0.18 SD for the infection trait FEC, reflecting our dominance estimates for rGIN-INF (0.08 to 0.30 SD).

We estimated low values of dominance (d = 0.003 to 0.01) for the trait EBV SCS for the three SNPs *ALCAMc.1017T>C*, in *ALCAMc.1104+10T>A* and *ALCAMc.1104+85T>C* in *ALCAM*. Accordingly, He et al. (2011) analysed low dominance effects in a range of d = -0.012 to 0.0008 for SCS considering the immune and inflammatory response related genes *CD4* and *STAT5b*. Negative (favourable) dominance effects were identified for the traits PERS and rFH-INF at the SNPs *ALCAMc.1104+10T>A* and *ALCAMc.1104+85T>C*, and for rFH-INF and Fat%, Pro% and FPR at all three loci. Dominance effects for the EBVs of Fat% and Pro% ranged from d = 0.02 to 0.05, which is in agreements with prior studies in Holstein cows focussing on the *DGAT1* gene (Bovenhuis et al., 2015; Kuehn et al., 2007; Pasandideh et al., 2015). Bovenhuis et al. (2015) and Szyda et al. (2014) identified altering *DGAT1* effects on milk production traits (milk yield, fat and protein content) throughout lactation. Accordingly, dominance effects for the *DGAT1* gene changed in dependence of the lactation number and were highest in first parity cows (Bovenhuis et al., 2015). The DSN cows in our study had in average 2.4 lactations, and dominance effects in our sample mostly representing younger cows, were quite small.

Conclusions

We identified three SNPs *ALCAMc.1017T>C, ALCAMc.73+32791A>G* and *ALCAMc.1104+85T>C* in the exon 9 and flanking intronic regions of the *ALCAM* gene via sequence analyses in an endangered small population of DSN cows by applying a selective

genotyping approach. Significant allele substitution effects for rFH-INF, rGIN-INF, EBV Fat%, EBV Pro% and EBV FPR were detected via logistic regression analysis. Genotype and allele frequencies significantly differed between *F. hepatica* infected and non-infected cows for all four SNP loci. Aiming on resistance against *F. hepatica*, favourable alleles were the G allele for the SNP *ALCAMc.73+32791A>G*, and the T alleles for the SNPs *ALCAMc.1017T>C* and *ALCAMc.1104+85T>C*. Allele substitution effects were antagonistic for rFH-INF and rGIN-INF for the SNPs *ALCAMc.1017T>C* and *ALCAMc.1104+85T>C*. Polymorphisms in the SNP *ALCAMc.1104+10T>A* revealed desirable effects for *F. hepatica* resistance, for PERS and for the EBVs of the test-day production traits Fat% and Pro%. Dominance effects were small for low heritable test-day traits, whereas dominance effects for rFH-INF were large and in the direction of desired resistance. Sequence analyses of the *ALCAM* gene combined with selective genotyping approaches detected additional polymorphisms influencing disease resistance. We suggest consideration of these SNPs on commercial SNP chips.

References

Agneessens J, Clearebout E, Dorny P, Borgsteede FHM, Vercruysse J. 2000. Nematode parasitism in adult dairy cows in Belgium. Veterinary Parasitology 90:83-92.

Alam MBB, Omar AI, Faruque MO, Notter DR, Periasamy K, Mondal MMH, Sarder MJU, Shamsuddin M, Cao J, Du X, Wu Z, Zhao S. 2019. Single nucleotide genes are significantly associated with resistance to *Haemonchus contortus* infection in goats. Journal of Animal Science and Biotechnology 10:30.

Ali AKA, Shook GE. 1980. An optimum transformation for somatic cell concentration in milk. Journal of Dairy Science 63:487-490.

Aliloo H, Pryce JE, González-Recio O, Cocks BG, Hayes BJ. 2016. Accounting for dominance to improve genomic evaluations of dairy cows for fertility and milk production traits. Genetics Selection Evolution 48:8.

Alvarez Rojas CA, Ansell BRE, Hall RS, Gasser RB, Young ND, Jex, AR, Scheerlinck J-PY. 2015. Transcriptional analysis identifies key genes involved in metabolism, fibrosis/tissue repair and the immune response against *Fasciola hepatica* in sheep liver. Parasites & Vectors 8: 124.

Bagheri M, Miraie-Ashtiani R, Moradi-Shahrbabak M, Nejati-Javaremi A, Pakdel A, von Borstel UU, Pimentel ECG, König S. 2013. Selective genotyping and logistic regression analyses to identify favorable SNP-genotypes for clinical mastitis and production traits in Holstein dairy cattle. Livestock Science 151:140-151.

Beesley NJ, Williams DJL, Paterson S, Hodgkinson J. 2017. *Fasciola hepatica* demonstrates high levels of genetic diversity, a lack of population structure and high gene flow: possible implications for drug resistance. International Journal of Parasitology 47:11-20.

Benavides MV, Weimer TA, Borba MFS, Berne MEA, Sacco AMS. 2009. Genetic analyses of polymorphisms on ovine chromosomes 5 and 20 and their effect on resistance to internal parasites. Small Ruminant Research 83:67-73.

Blanco-Penedo I, Höglund J, Fall N, Emanuelson U. 2012. Exposure to pasture borne nematodes affects individual milk yield in Swedish dairy herds. Vet Parasitol 188:93-98.

Bolormaa S, Pryce JE, Zhang Y, Reverter A, Barendse W, Hayes BJ, Goddard ME. 2015. Non-additive genetic variation in growth, carcass and fertility traits of beef cattle. Genetics Selection Evolution 47:26.

Borgsteede FHM, Tibben J, Cornelissen JBWJ, Agneessens J, Gaasenbeek CPH. 2000. Nematode parasites of adult dairy cattle in the Netherlands. Veterinary Parasitology 89:287-296.

Bovenhuis H, Visker MHPW, van Valenberg HJF, Buitenhuis AJ, Arendok JAM. 2015. Effects of the *DGAT1* polymorphism on test-day milk production traits throughout lactation. Journal of Dairy Science 98:6572-6582.

Brady MT, O'Neill SM, Dalton JP, Mills KHG. 1999. *Fasciola hepatica* suppresses a protective Th1 response against *Bordetella pertussis*. Infection and Immunity 67:5372-5378.

Calus MPL, Berry DP, Banos G, de Haas Y, Veerkamp RF. 2013. Genomic selection: the option for new robustness traits? Advances in Animal Biosciences 4:618-625.

Caminade C, van Dijk J, Baylis M, Williams D. 2015. Modelling recent and future climatic suitability for fasciolosis in Europe. Geospatial Health 9: 301-308.

Charlier J, van der Voort M, Kenyon F, Skuce P, Vercruysse J. 2014. Chasing helminths and their economic impact on farmed ruminants. Trends in Parasitology 30:361-367.

Charon KM, Moskwa B, Rutkowski R, Gruszczyńska J, Świderek W. 2002. Microsatellite polymorphism in DBR1 gene (MHC class II) and its relation to nematode faecal egg count in Polish Heath Sheep. Journal of Animal and Feed Sciences 11:47-58.

Coppieters W, Mes THM, Druet T, Farnir F, Tamma N, Schrooten C, Cornelissen AWCA, Georges M, Ploeger HW. 2009. Mapping QTL influencing gastrointestinal nematode burden in Dutch Holstein-Friesian dairy cattle. BMC Genomics 10:96.

Czarnik U, Galiński M, Pareek CS, Zabolewicz T, Wielgosz-Groth Z. 2007. Study of an association between SNP 775C>T within the bvine ITBG2 gene and milk performance traits in Black and White cows. Czech Journal of Animal Science 52:1-6.

Duarte S, Baber J, Fujii T, Coito AJ. 2015. Matrix metalloproteinases in liver injury, repair and fibrosis. Matrix Biology 44-46:147-156.

Dusza M, Pokorska J, Mukulska J, Kulaj D and Cupial M. 2018. *L-Selectin* gene polymorphism and its association with clinical mastitis, somatic cell score, and milk production in Polish Holstein-Friesian cattle. Czech Journal of Animal Science 63:256-262.

Falconer DS and Mackay TFC. 1996. Introduction to Quantitative Genetics. 4th ed. Longmans Green, Harlow, Essex, UK.

Foster N, Elsheikka HM. 2012. The immune response to parasitic helminths of veterinary importance and its potential manipulation for future vaccine control strategies. Parasitology Research 110:1587-1599.

Gengler N. 1996. Persistency of lactation yields: a review. Interbull Bull 12:87-96.

Georges M, Charlier C, Hayes B. 2019. Harnessing genomic information for livestock improvement. Nature Reviews Genetics 20:135-156.

Gray GD. 1997. The use of genetically resistant sheep to control nematode parasitism. Veterinary Parasitology 72:345-357.

Han YP. 2006. Matrix metalloproteinases, the pros and cons, in liver fibrosis. Journal of Gastroenterology and Hepatology 21 Suppl. 3:88-91.

Hanotte O, Ronin Y, Agaba M; Nilsson P, Gelhaus A, Horstmann R, Sugimoto Y, Kemp S, Gibson J, Korol A, Soller M, Teale A. 2002. Mapping of quantitative trait loci controlling trypanotolerance in a cross of tolerant West African N'Dama and susceptible East African Boran cattle. Proceedings of the National Academy of Sciences of the United States of America 100:7443-7448.

He Y, Chu Q, Ma P, Wang Y, Zhang Q, Sun D, Zhang Y, Yu Y, Zhang Y. 2011. Association of bovine *CD4* and *STAT5b* single nucleotide polymorphisms with somatic cell scores and milk production traits in Chinese Holsteins. Journal of Dairy Research 78:242-249.

Henshall JM, Goddard ME. 1999. Multiple-trait mapping of quantitative trait loci after selective genotyping using logistic regression. Genetics 151:885-894.

Heriazon A, Quinton M, Miglior F, Leslie KE, Sears W, Mallard BA. 2013. Phenotypic and genetic parameters of antibody and dalyed-type hypersensitivity responses of lactating Holstein cows. Veterinary Immunology and Immunopathology 154:83-92.

Hill WG, Robertson A. 1968. Linkage disequilibrium in finite populations. Theoretical and Applied Genetics 38:226-231.

Hill WG, Goddard ME, Visscher PM. 2008. Data and theory point to mainly additive genetic variance for complex traits. PLoS Genetics 4(2):e1000008.

Hillyer GV, Soler de Galanes M, Buchón P, Bjorland J. 1996. Herd evaluation by enzyme-linked immunosorbent assay for the determination of *Fasciola hepatica* infection in sheep and cattle from Altiplano of Bolivia. Veterinary Parasitology 61:211-220.

Huth F. 1995. Laktation des Rindes. Verlag Eugen Ulmer, Stuttgart, Germany.

Ingale SL, Singh P, Raina OK, Verma AK, Channappanavar R, Mehra UR. 2010. Interleukin-2 and interleukin-10 gene expression in calves experimentally infected with *Fasciola gigantica*. Livestock Science 131:141-143.

Kimura K, Goff JP, Kehrli ME. 1999. Effects of the presence of the mammary gland on expression of neutrophil adhesion molecules and myeloperoxidase activity in periparturient dairy cows. Journal of Dairy Science 82:2385-2392.

King JA, Ofori-Acquah SF, Stevens T, Al-Mehdi A-B, Fodstad O, Jiang WG. 2004. Activated leukocyte cell adhesion molecule in breast cancer: prognostic indicator. Breast Cancer Research 6:478-487.

Klein S-L, Scheper C, Brügemann K, Swalve HH, König S. 2019. Phenotypic relationships, genetic parameters, genome-wide associations, and identification of potential candidate genes for ketosis and fat-to-protein ratio in German Holstein cows. Journal of Dairy Science 102:6276-6287.

Koeck A, Jamrozik J, Schenkel FS, Moore RK, Lefebvre DM, Kelton DF, Miglior F. 2014. Genetic analysis of milk ß-hydroxybutyrate and its association with fat-to-protein ratio, body condition score, clinical ketosis, and displaced abomasums in early first lactation of Canadian Holsteins. Journal of Dairy Science 97:7286-7292.

König S, Chang YM, von Borstel UU, Gianola D, Simianer H. 2008. Genetic and phenotypic relationships among milk urea nitrogen, fertility, and milk yield in Holstein cows. Journal of Dairy Science 91:4372-4382.

Konno A, Ahn JS, Kitamura H, Hamilton MJ, Gebe JA, Aruffo A, Davis WC. 2001. Tissue distribution of CD6 and CD6 ligand in cattle: expression of the CD6 ligand (CD166) in the autonomic nervous system of cattle and the human. Journal of Leukocyte Biology 69:944-950.

Kordalis NG, Arsenopoulos K, Vasileiou NGC, Mavrogianni VS, Lianou DT, Papadopoulos E and Fthenakis GC. 2019. Field evidence for association between increased gastrointestinal nematode burden and subclinical mastitis in dairy sheep. Veterinary Parasitology 265:56-62.

Korkuć P, Arends D, Scheper C, May K, König S, Brockmann GA. 2017. 1-step versus 2-step imputation: a case study in German Black Pied cattle. Proceedings of the World Congress on Genetics Applied to Livestock Production 11:282.

Kozat S, Denizhan V. 2010. Glucose, lipid, and lipoprotein levels in sheep naturally infected with *Fasciola hepatica*. Journal of Parasitology 96:657-659.

Kuehn C, Edel C, Weikard R, Thaller G. 2007. Dominance and parent-of-origin effect of coding and non-coding alleles at the acylCoA-diacylglycerol-acyltransferase (DGAT1) gene on milk production traits in German Holstein cows. BMC Genetics 8:62.

Kuerpick B, Schnieder T, Strube C. 2012. Seasonal pattern of *Fasciola hepatica* antibodies in dairy herds in Northern Germany. Parasitology Research 111:1085-1092.

Lei Z, Yuan-Yuan Y, Zhong-Hao L, Li-Juan K, Guan-Dong X, He-Shuang D, Gen-Liu W. 2006. Detection and characterization of PCR-SSCP markers of the bovine lactoferrin gene for clinical mastitis. Asian-Australasian Journal of Animal Science 19:1399-1403.

Lucena AN, Cuartero LG, Mulcahy G, Zintl A. 2017. The immunomodulatory effects of co-infection with *Fasciola hepatica*: From bovine tuberculosis to Johne's disease. The Veterinary Journal 222: 9-16.

Lunter PC, van Kilsdonk JWJ, van Beek H, Cornelissen IMHA, Bergers M, Willems PHGM, van Muijen GNP, Swart GWM. 2005. Activated Leukocyte Cell Adhesion Molecule (ALCAM/CD166/MEMD), a novel actor in invasive growth, controls matrix metalloproteinase activity. Cancer Research 65:19.

Madsen P, Jensen J. 2013. A user's guide to DMU. A package for analysing multivariate mixed models. Version 6, release 5.2. Center for quantitative genetics and genomics. Dept. Of Molecular Biology and Genetics, University of Aarhus, Reserach Centre Foulum. Tjele, Denmark.

Marshall, K, Mugambi JM, Nagda S, Sonstegard TS, Van Tassell CP, Baker RL, Gibson JP. 2013. Quantitative trait loci for resistance to Haemonchus contortus artificial challange in Red Maasai and Dorper sheep of East Africa. Animal Genetics 44:285-295.

May K, Brügemann K, König S, Strube C. 2017. Patent gastrointestinal nematode infections in organically and conventionally pastured dairy cows and their impact on individual milk and fertility parameters. Veterinary Parasitology 245:119-127.

May K, Scheper C, Brügemann K, Yin T, Strube C, Korkuć P, Brockmann GA, König S. 2019. Genome-wide associations and functional gene analyses for endoparasite resistance in an endangered population of native German Black Pied cattle. BMC Genomics 20:227.

McClure J, Cromie AR, Sayers R, Graham D, Byrne N, Berry DP. 2014. Genetics of susceptibility to bovine diarrhea virus, bovine herpesvirus type 1 and *Fasciola hepatica* in cattle. Proceedings, 10th World Congress of Genetics Applied to Livestock Production.

Mendes EA, de Oliveira Mendes TA, dos Santos SL, Menezes-Souza D, Bartholomeu DC, Martins IV, Silva LM, Lima Wdos S. 2013. Expression of IL-4, IL-10 and IFN-γ in the liver tissue of cattle that are naturally infected with *Fasciola hepatica*. Veterinary Parasitology 195:177-182.

Mezo M, Gonzalez-Warleta M, Antonio Castro-Hermida J, Muino L, Ubeira FM. 2011. Association between anti-*F. hepatica* antibody levels in milk and production losses in dairy cows. Veterinary Parasitology 180:237-242.

Oviedo-Boyso J, Valdez-Alarcón, Cajero-Juárez M, Ochoa-Zarzosa A, López-Meza JE, Bravo-Patiño, Baizabal-Aguirre VM. 2007. Innate immune response of bovine mammary gland pathogenic bacteria responsible for mastitis. Journal of Infection 54:399-409.

Pasandideh M, Mohammadabadi MR, Esmailizadeh AK, Tarang A. 2015. Association of bovine PPARGC1A and OPN genes with milk production and composition in Holstein cattle. Czech Jorunal of Animal Science 60:97-104.

Perkins KH, VandeHaar MJ, Tempelman RJ, Burton JL. 2001. Negative energy balance does not decrease expression of leukocyte adhesion or antigen-presenting molecules in cattle. Journal of Dairy Science 84:421-428.

Phiri IK, Phiri AM, Harrison LJS. 2007. The serum glucose and ß-hydroxybutyrate levels in sheep with experimental *Fasciola hepatica* and *Fasciola gigantica* infection. Veterinary Parasitology 143:287-293.

Piedrafita D, Raadsma HW, Prowse R, Spithill TW. 2004. Immunology of the host-parasite relationship in fasciolosis (*Fasciola hepatica* and *Fasciola gigantica*). Canadian Journal of Zoology 82:233-250.

Pryce JE, Haile-Mariam M, Verbyla K, Bowman PJ, Goddard ME, Hayes BJ. 2010. Genetic markers for lactation persistency in primiparous Australian dairy cows. Journal of Dairy Science 93:2202-2214.

R Core Team. 2013. R. A language and environmental for statistical computing. R Core Team, Vienna, Austria.

Rajala-Schultz PJ, Saville WJA, Frazer GS, Wittum TE. 2001. Asscoiation between milk urea nitrogen and feritlity in Ohio Dairy cows. Journal of Dairy Science 84:482-489.

Ren G, Roberts AI, Shi Y. 2011. Adhesion molecules. Cell Adhesion & Migration 5:1.

Roy B, Brahma B, Ghosh S, Pankaj PK and Mandal G. 2011. Evaluation of milk urea concentration as useful indicator for dairy herd management: a review. Asian Journal of Animal and Veterinary Advances 6:1-19.

Ruiz-Campillo MT, Molina Hernandez V, Escamilla A, Stevenson M, Perez J, Martinez-Moreno A, Donnelly S, Dalton JP, Cwiklinski K. 2017. Immune signatures of pathogenesis in the peritoneal compartment during early infection of sheep with *Fasciola hepatica*. Scientific Reports 7:2782.

SAS Institute Inc. 2013. Version 9.4. SAS Institute, Inc., Cary, NC.

Schwaiger F-W, Gostomski D, Stear MJ, Duncan JL, McKellar QA, Epplen JT, Buitkamp J. 1995. An ovine *Major Histocompatibility Complex DRB1* allele is associated with low faecal egg counts following natural, predominantly *Ostertagia circumcincta* infection. International Journal of Parasitology 25:815-822.

Schweizer G. 2005. Estimating the financial losses due to bovine fasciolosis in Switzerland. Veterinary Record 157:188-193.

Sun C, VanRaden PM, Cole JB, O'Connell JR. 2014. Improvement of prediction ability for genomic selection of dairy cattle by including dominance effects. PlosOne 9:e103934.

Swart GWM. 2002. Activated leukocyte cell adhesion molecule (CD166/ALCAM): Developmental and mechanistic aspects of cell clustering and cell migration. European Journal of Cell Biology 81:313-321.

Szyda J, Komisarek J, Antkowiak I. 2014. Modelling effects of candidate genes on complex traits as variables over time. Animal Genetics 45:322-328.

Tao W, Mallard B, Karrow N, Bridle B. 2003. Construction and application of a bovine immune-endocrine cDNA microarray. Veterinary Immunology and Immunopathology 101:1-17.

Thienpont D, Rochette F, van Parijs OFJ. 1979. Diagnosing helminthiasis by coprological examination. Janssen Research Foundation, Beerse, Belgium, pp. 187.

Thompson-Crispi KA, Sewalem A, Miglior F, Mallard BA. 2012. Genetic parameters of adaptive immune response traits in Candian Holsteins. Journal of Dairy Science 95:401-409.

Tilquin P. 2003. Methodological aspects of the mapping of disease resistance in livestock. Louvain-la-Neuve, Belgiuem: Université catholique de Louvain, PhD thesis.

Twomey AJ, Carroll RI, Doherty ML, Byrne N, Graham DA, Sayers RG, Blom A, Berry DP. 2018. Genetic correlations between endo-parasite phenotypes and economically important traits in dairy and beef cattle. Journal of Animal Science 96:407-421.

Twomey AL, Berry DP, Evans RD, Doherty ML, Graham DA, Purfield DC. 2019. Genome-wide association study of endo-parasite phenotypes using imputed whole-genome sequence data in dairy and beef cattle. Genetics Selection Evolution 51:15.

Untergasser A, Nijveen H, Rao X, Bisseling T, Geurts R, Leunissen JAM. 2007. Primer3Plus, an enhanced web interface to Primer3. Nucleic Acids Res 35:71-74.

Vercruysse J, Charlier J, van Dijk J, Morgan ER, Geary T, von Samson-Himmelstjerna G, Claerebout E. 2017. Control of helminth infections by 2030. Parasitology 145:1-10.

Wolfinger R, O'Connel M. 1993. Generalized linear mixed models: a pseudo-likelihood approach. Journal of Statistical Computation and Simulation 48:233-243.

Walker SM, Johnston C, Hoey EM, Fairweather I. 2011. Population dynamics of the liver fluke, *Fasciola hepatica*: the effect of time and spatial separation on the genetic diversity of fluke populations in the Netherlands. Parasitology 138:215-223.

Weidle UH, Eggle D, Klostermann S, Swart GWM. 2010. ALCAM/CD166: Cancer-related Issues. Cancer Genomics Proteomics 7:231-244.

Wagner M, Bilinska M, Pokryszko-Dragan A, Sobczynski M, Cyrul M, Kusnierczyk P, Jasek M. 2014. *ALCAM* and CD6 – multiple sclerosis risk factors. Journal of Neuroimmunology 276:98-103.

Walker SM, Johnston C, Hoey EM, Fairweather I, Borgsteede F, Gaasenbeek C, Prodöhl PA, Trudgett A. 2011. Population dynamics of the liver fluke, *Fasciola hepatica*: the effect of time and spatial separation on the genetic diversity of fluke populations in the Netherlands. Parasitology 138:215-223.

Zhang FK, Hou JL, Guo AJ, Tian AL, Sheng ZA, Zheng WB, Huang WY, Elsheikka HM, Zhu XQ. 2018. Expression profiles of genes involved in TLRs and NLRs signaling pathways of water buffaloes infected with *Fasciola gigantica*. Molecular Immunology 94:18-26.

Zheng M, Zhang L, Yu Hongsong, Hu J, Cao Q, Huang G, Huang Y, Yuan G, Kijlstra A, Yan P. 2016. Genetic polymorphisms of cell adhesion molecules in Behcet's disease in a Chinese Han population. Scientific Reports 6:24974.

Zhou P, Du L-F, Lv G-Q, Yu X-M, Gu Y-L, Li J-P, Zhang C. 2011. Functional polymorphisms in *CD166/ALCAM* gene associated with increased risk for breast cancer in a Chinese population. Breast Cancer Research and Treatment 128:527-534.

KAPITEL 5

Fasciola hepatica seroprevalence in Northern German dairy herds and associations between bulk tank milk antibody levels with milk production parameters and milk ketone bodies ß-hydroxybutyrate and acetone

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Abstract

Infections with the liver fluke *Fasciola hepatica* remain a serious problem in dairy herds causing significant production losses. In sheep, a strong relationship between *F. hepatica* infections and an increase in serum ketone bodies due to reduced feed intake and liver damage was demonstrated. We hypothesized that *F. hepatica* infections might contribute to an increase in milk ketone bodies in dairy herds. Thus, the objective of the study was to estimate the association between *F. hepatica* bulk tank milk (BTM) antibodies and milk production parameters (milk yield, milk protein, fat yield), somatic cell count (SCC) and the milk ketone bodies ß-hydroxybutyrate (BHB) and acetone, inferred from Fourier transform infrared (FTIR) spectrometry, via linear mixed model analysis. A further aim was to follow up the *F. hepatica* seroprevalence in dairy herds in the northern German region East Frisia. We collected BTM samples between October and December from 1022 herds in 2017 and 1318 herds in 2018. Overall, 33.1% of the herds were tested positive in 2017 and 37.0% in 2018, showing decreased *F. hepatica* seroprevalences compared to prior seroprevalence studies in the same region in 2010, 2008 and 2006 (> 45% positive herds). We estimated a significant negative association ($P < 0.0001$) between herd *F. hepatica* infection category and average milk yield with a loss of -1.62 kg per cow per day in strongly infected herds compared to BTM ELISA negative herds. Moreover, *F. hepatica* infection category had a significant effect on herd average milk protein and fat yield ($P < 0.0001$), showing a decrease of 0.06 kg for both parameters from BTM ELISA negative herds to strongly infected herds. No significant association with milk SCC was found. Regarding ketone bodies, we estimated significant higher average BHB values in strongly infected herds compared to the other three infection categories in the model analysis ($P = 0.002$). The association between *F. hepatica* infection category and acetone values was not significant. Besides primary ketosis, fasciolosis should be considered as differential diagnosis in dairy herds with increased BHB values.

Keywords:

Fasciolosis; liver fluke; milk production; somatic cell count; linear mixed models; dairy cows; Germany

Introduction

Fasciolosis, caused by the liver fluke *Fasciola hepatica,* is a widespread parasitic disease causing high economic losses in livestock production worldwide (Mehmood et al., 2017). Seroprevalences range between 7.1% and 93.0% in European dairy herds, depending on geographical region (Byrne et al., 2018; Höglund et al., 2010). Kuerpick et al. (2013) evaluated *F. hepatica* bulk-tank milk (BTM) seroprevalences in different German federal states in 2008 and identified the highest seroprevalences in the northern federal states Schleswig-Holstein (38.4%) and Lower Saxony (29.4%). In a multi-year study performed in East Frisia, a coastal region in Lower Saxony with suspected high liver fluke exposure coupled with a high proportion of grazing cattle, BTM seroprevalences ranged even between 45.1% and 57.1%, depending on the sampling year and month (2006, 2008, 2010; January, September, November), respectively (Kuerpick et al., 2012a; Kuerpick et al., 2012b).

Financial losses due to fasciolosis amount to 30 to 299 € per infected cow (Charlier et al., 2007, 2009; Schweizer et al., 2005). These losses are largely arising from reduced milk and meat production and reduced fertility due to fasciolosis, but additionally secondary bacterial infections due to immunosuppression during *F. hepatica* infections contribute to economic losses (Brady et al., 1999; Lucena et al., 2017). Studies based on herd antibody levels reported decreases in the annual average milk yield up to 4.2 kg per cow and day in infected herds (Charlier et al., 2007; Howell et al., 2015; Köstenberger et al., 2017; Mezo et al., 2011). Moreover, Charlier et al. (2012) estimated a significant increase of 303 kg in the 305-day milk production in treated dairy herds compared to the untreated control groups. Increases in herd *F. hepatica*-antibody levels were associated with reductions between 0.06 to 0.09% in milk fat content and 0.05% in milk protein content (Charlier et al., 2007; Köstenberger et al., 2017). However, this effect was not observed in other studies (Charlier et al., 2012; Howell et al., 2015; Mezo et al., 2011). Howell et al. (2015) and May et al. (2019) hypothesized increased udder bacterial infections, and thus an increase in milk somatic cell count (SCC) due to immunodepression in *F. hepatica* infected cows. However, both studies estimated no significant relationship between *F. hepatica* infections and somatic cells in milk.

Studies in sheep demonstrated a strong association between patent *F. hepatica* infections and a decrease in the serum glucose level (Kozat and Denizhan, 2010; Phiri et al., 2007; Yüksek et al., 2013). This relationship is explained by a reduced feed intake and liver damage due to migrating flukes, contributing to a reduction of liver glycogen. In consequence, free fatty acids and ketone bodies in serum increase (Phiri et al., 2007). Ketone bodies (ß-hydroxybutyrate

[BHB], acetone, acetoacetone) are routinely used biomarkers to detect hyperketonemia and primary ketosis in dairy cows (Koeck et al., 2014). Especially in early lactation, dairy cows enter a state of negative energy balance due to the imbalance in the supply and demand of glucose (Baird, 1982), resulting in an increased incidence of primary ketosis (Gaddis et al., 2018). Measuring BHB in serum or plasma using enzymatic laboratory methods is the gold standard for the diagnosis of hyperketonemia and primary ketosis (Pineda and Cardoso, 2015). Additionally, BHB and acetone can be measured in milk by flow-injection analysis and Fourier transform infrared (FTIR) spectrometry. Prediction values of BHB from routine milk test-day recording by using FTIR spectrometry were introduced in several countries into monitoring programs to reduce hyperketonemia and ketosis in dairy herds (e.g., Ketoscreen milk BHB test, CanWest DHI, Guelph, ON, Canada; Ketodetect, CLASEL, France; Schwarz et al., 2015). Chandler et al. (2018) and Renaud et al. (2019) estimated an accuracy of 64.0% to 92.9% and a sensitivity of 81% for milk BHB predicted by FTIR spectrometry for diagnosing primary ketosis. They concluded that this technique can be used as a herd-level monitoring tool to assess ketosis in dairy herds. However, phenotypic correlations between BHB predicted from milk infrared spectra or blood BHB with producer recorded ketosis scenarios are very low (0.02 to 0.19; Belay et al., 2017; Koeck et al., 2014). Trembley et al. (2018) identified high BHB values in non-ketotic cows due to metabolic adaptation. Furthermore, increased blood or milk ketone bodies in dairy cows might be explained by metabolic alterations and changes in feed intake in response to *F. hepatica* infections as it was proven in sheep (Phiri et al., 2007).

Therefore, we aimed to investigate the association between herd *F. hepatica* antibody levels and the content of milk ketone bodies BHB and acetone predicted by FTIR spectrometry. Furthermore, we assessed the association between herd *F. hepatica* antibody levels with monthly recorded herd-level milk production parameters. A third objective of the study was to follow-up the *F. hepatica* seroprevalence in dairy herds in the northern German region East Frisia.

Material and Methods

Study area and bulk tank milk samples

The study was conducted in East Frisia, a coastal region in the northern German federal state Lower Saxony. The average number of cows per herd was 110.7 in the year 2017 and 114.1 in 2018. For all participating herds, Holstein-Frisian was the main breed. In total, we examined 2340 BTM samples collected between 25th October and 27th December 2017 and between 23th

October and 24th December 2018 for the presence of antibodies against *F. hepatica*. Of all BTM samples in 2017, 16.1% were assessed in October, 78.9% in November and 5.1% in December. In 2018, 22.4% of BTM samples were collected in October, 72.2% in November and 5.5% in December. Results of *F. hepatica* antibody levels were available for 1541 herds, including 1022 herds in 2017 and 1318 herds in 2018. Repeated BTM ELISA results were available for 799 herds, while 742 herds were tested for *F. hepatica* antibody levels in one year (2017 or 2018) only.

Milk production parameters and milk ketone bodies

Herd data and test-day production data were provided from the National Genetic Evaluation Center (Vereinigte Informationssysteme Tierhaltung [VIT], Verden, Germany). Test-day herd data from monthly routine milk recording included the number of recorded cows, the average days in milk (DIM), the average lactation number and the averages for milk yield (in kg), milk protein yield (in kg), milk fat yield (in kg) and SCC (cells per ml) for each test-day record. Test-day production data were available for 1535 herds including records between December 2016 and December 2018. The content of milk ketone bodies BHB and acetone was measured in mmol/L during routine milk recording via FTIR spectrometry (MilkoScan FT60, FOSS, Hillerød, Denmark). The ketone body dataset included individual cow BHB and acetone records and individual cow information on lactation number and DIM for each test-day date between January 2017 and May 2019. Individual cow BHB and acetone records were available for 1514 herds.

Bulk tank milk ELISA

BTM samples of dairy herds from routine milk recording were tested for *F. hepatica* antibodies using the commercially available IDEXX Fasciolosis Verification test ELISA kit (Montpellier, France; formerly Pourquier®ELISA Bovine fasciolosis serum and milk verification) in the laboratory of the state control association for milk recording (*Landeskontrollverband* [LKV] Weser-Ems,Leer, Germany). All BTM samples were preserved with boric acid before analysis. The samples were analyzed according to the manufacturer's instructions, i.e. testing the coated well with the provided antigen (f2-antigen, a fraction purified from E/S-antigen of *F. hepatica*) against a second uncoated well. The resulting absorbance from the uncoated well was subtracted from the measured absorbance of the f2-antigen-coated well. Based on the ratio of the absorbance value of the positive samples (S) to the mean absorbance value of the positive

control (P), results were expressed as sample to positive percentage (S/P%) for each sample. BTM ELISA results were considered positive in case of S/P% > 30% (cf. Table 1).

Data analyses

Statistical analyses were performed using SAS version 9.4 (SAS Institute; Cary, NC, USA). *P*-values ≤ 0.05 were regarded as significant for all analyses. We used the PROC FREQ procedure for descriptive statistics and to analyze differences in herd infection categories between the sampling years. We tested for differences in the S/P% values between the year 2017 and 2018 by using a Wilcoxon matched-pairs test.

The dataset for the association analysis between *F. hepatica* BTM ELISA results with herd milk production parameters (milk yield, protein yield, fat yield, SCC) included all herd averages of test-day records for the milk production parameters within the month of the ELISA examination and five months before (e.g., test-day records between June and November 2018 for BTM ELISA result in November 2018). Herd test-day records with lower than ten recorded cows, records with extremely low values for milk yield (≤ 10 kg) and test-day records with a herd average of more than 250 DIM were excluded from the corresponding year. Afterwards, herds with less or equal than two test-day records within one year were excluded from the corresponding year as well. After data processing, the average number of test-day records per herd was 5.7 in 2017 (range: 3.0-8.0 records) and 5.5 (range: 4.0-7.0) in 2018. To investigate the association between *F. hepatica* BTM ELISA results and herd average milk BHB and acetone values, only observations of cows between DIM 5 and 60 were included in the analysis. Herd average BHB and acetone values (in µmol/L) were computed for each herd test-date including the month of BTM ELISA result and the three months before and after BTM ELISA (e.g., test-day records between August 2018 and February 2019 for BTM ELISA result in November 2018). The average DIM and average lactation number were computed for all cows with BHB and acetone records between DIM 5 and 60 for each test-day record within each herd. Herds with less or equal than two test-day records within one year were excluded from the corresponding year. For the ketone body dataset, the average number of test-day records per farm was 6.6 in 2017 (range: 3.0-9.0 records) and 6.5 (range: 3.0-9.0) in 2018. We computed herd averages (e.g., ketone bodies, DIM) based on 190,772 cow records (67,985 in 2017/2018; 122,787 in 2018/2019) between DIM 5 and 60.

Table 1. Results and interpretation of the IDEXX Fasciola Verification ELISA. CI = Confidence interval.

S/P% of the sample	Proportion of animals infected within the herd	BTM status	Prevalence in 2017	Prevalence in 2018
S/P% ≥ 150%	Strong infection (> 50% of herd infected)	Positive	17.61% (180/1022) (95% CI: 15.32-20.09%)	19.12% (252/1318) (95% CI: 17.03-21.35%)
80 < S/P% < 150%	Medium infection (20%-50% of herd infected)	Positive	7.14% (73/1022) (95% CI: 5.64-8.90%)	8.04% (106/1318) (95% CI: 6.63-9.64%)
30 < S/P% ≤ 80%	Low infection (< 20% of herd infected)	Positive	8.32% (85/1022) (95% CI: 6.70-10.18%)	9.79% (129/1318) (95% CI: 8.24-11.52%)
S/P% ≤ 30%	No or very weak infection	Negative	66.93% (684/1022) (95% CI: 63.95-69.81%)	63.05% (831/1318) (95% CI: 60.38-65.66%)

We applied a linear mixed model to study the association between the dependent variables (herd average test-day milk yield, protein yield, fat yield, SCC, BHB, acetone) and the independent variable *F. hepatica* herd infection category (strong, medium, low, no or weak infection). *F. hepatica* herd infection category and the combination of month and year for each test-day record were included as fixed effects in the model. Further covariables included in the model were the average DIM and the average lactation number for each test-day. The herd was modelled as a random effect. Fixed effects and covariables in the models were tested for significance by stepwise selection. The herd size was not statistically significant in the models for the milk production parameters and for milk ketone bodies (BHB, acetone), and was therefore removed from the models. We selected the model with the smallest Akaike information criteria (AIC) as the best model. For model validation, we checked the normal distribution of residuals by QQ plots. We estimated least-squares means for *F. hepatica* herd infection categories, and we tested the differences with the Bonferroni adjustment.

Results

Fasciola hepatica *BTM ELISA results*

In total, 2430 BTM samples were analysed for *F. hepatica* antibodies. Of the 1022 herds investigated in 2017, 33.1% (338/1022; 95% confidence interval [CI]: 30.2-36.1%) showed a positive BTM result. In 2018, 37.0% (487/1318; 95% CI: 34.3-39.6%) of the 1318 samples were tested positive. From the 799 herds with repeated ELISA measurements, 310 herds (38.8%) were positive. Of these 310 herds, 76.8% (238/310; 95% CI: 71.7-81.4%) were positive in both years and 23.2% (72/310; 95% CI: 18.6-28.3%) in one sampling year only. Of all herds being positive in one year only, 58.3% (42/72; 95% CI: 46.1-69.9%) switched from a negative status in 2017 to a positive status in 2018, while the remaining 41.7% (30/72; 95% CI: 30.2-53.9%) of the herds showed the opposite effect.

Regarding infection categories, 432 (18.5%) of the 2430 samples were in the high infection category, 179 (7.6%) in the medium infection category and 214 (9.2%) in the low infection category, while 1515 (64.7%) were assigned to a BTM ELISA negative herd status. Table 1 presents detailed results on the infection categories according to the sampling year. The S/P% values for BTM samples ranged between -24.44-384.22 S/P% in 2017 and between -1.91-251.53 S/P% in 2018 (Figure 1). The mean S/P% was 55.08 S/P% in 2017 and 56.05 S/P% in 2018 with an overall mean of 55.62 S/P%. We identified no significant difference in S/P%

values between BTM results in 2017 and 2018 (Wilcoxon matched-pairs signed rank test; P = 0.257).

Milk production parameters and milk ketone bodies

Table 2 presents descriptive statistics for herd data, average herd milk production parameters and average herd BHB and acetone values used in the model analysis. For milk production parameters, we used 11,687 average test-day records from 1464 herds in the multivariable analyses. Herd averages for milk production parameters were 26.2 kg per cow per test-day for milk yield (range: 10.1-42.7 kg), 0.9 kg per cow per test-day for protein yield (range: 0.3-1.4), 1.0 kg per cow per test-day for fat yield (range: 0.4-1.7) and 237.2 somatic cells per ml (x 1,000) (range: 29.0-970.0).

The milk ketone body dataset included 13,565 records for the average herd BHB content and 13,235 records for the average herd acetone content from 1433 herds (Table 2). The average BHB content was 51.8 µmol/L (range: -90.0-950.0 µmol/L) and the average acetone content 51.9 µmol/L (range: -160.0-1490.0 µmol/L). For the 190,772 individual cows recorded between DIM 5 and 60 (basis dataset for herd averages), milk BHB values ranged between -470.0 and 2490 µmol/L with a mean of 48.6 µmol/L and milk acetone values ranged between -550.0 and 3010 µmol/L with a mean of 40.0 µmol/L.

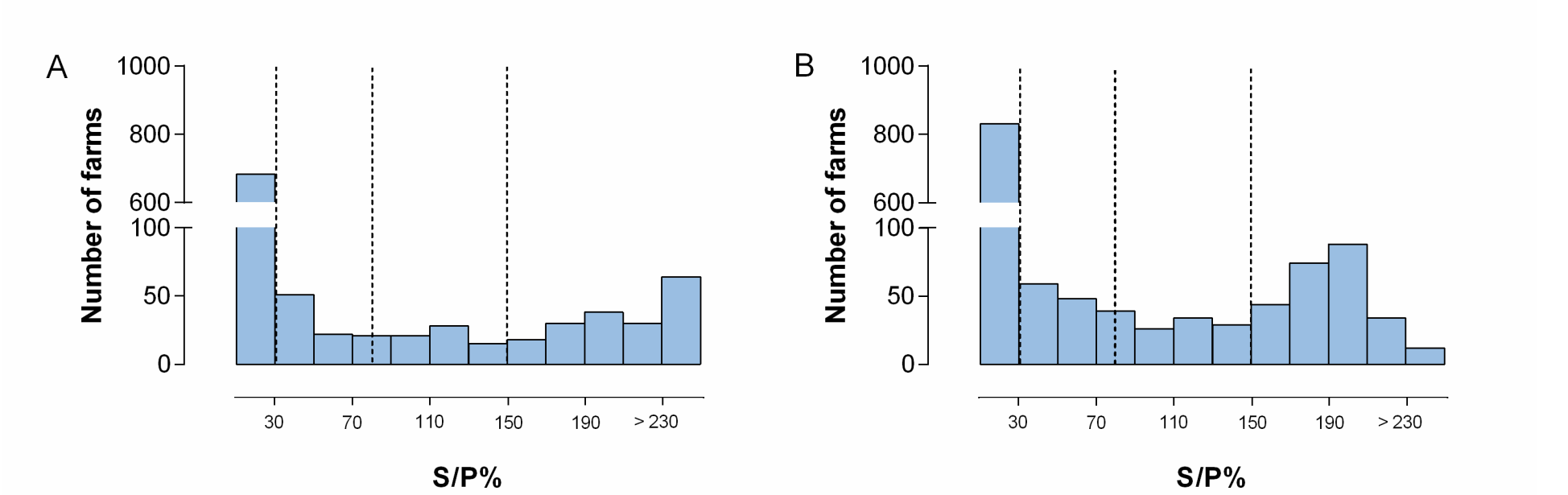

Figure 1. Frequency histogram of *F. hepatica* BTM ELISA results expressed as S/P% values from dairy herds in the northern German region East Frisia in 2017 (A) and 2018 (B). Dashed lines represent the cut-off values for different herd infection categories: S/P% ≤ 30% = no or very weak herd infection (BTM ELISA negative); 30 < S/P% ≤ 80% = low herd infection; 80 < S/P% < 150% = medium herd infection; S/P% ≥ 150% = strong herd infection.

Table 2. Descriptive statistics for herd data, average herd milk production parameters and average herd milk ß - hydroxybutyrate (BHB) and acetone values used in the model analysis.

Variable	Records	Herds	Mean	SD	Range
Test - day milk production dataset					
Average lactation number	11,678	1464	2.7	0.4	1.0 - 7.0
Average days in milk	11,678	1464	189.3	26.1	66.0 - 250.0
Average milk yield (kg)	11,678	1464	26.2	4.5	10.1 - 42.7
Average protein yield (kg)	11,678	1464	0.9	0.2	0.3 - 1.4
Average fat yield (kg)	11,678	1464	1.0	0.2	0.4 - 1.7
Average somatic cell count (x 1,000)	11,678	1464	237.2	114.1	29.0 - 970.0
Test - day milk ketone body dataset[1]					
Average lactation number	13,565	1433	2.9	0.9	1.0 - 10.0
Average days in milk	13,565	1433	31.1	7.9	5.0 - 59.0
Average BHB (µmol/L)[2]	13,565	1433	51.8	36.8	- 90.0 - 950
Average acetone (µmol/L)[2]	13,235	1433	51.9	77.3	- 160.0 - 1490.0

SD, standard deviation.

[1]Only cows between DIM 5 and 60 were included in the analyses.

[2]Values predicted from FTIR spectrometry.

Association between* F. hepatica *BTM ELISA results and milk production parameters

Table 3 shows least-squares means and results of the test of significance for fixed effects (sum of squares type III) from the linear mixed model analysis for milk production parameters. The association between *F. hepatica* herd infection category and milk yield was significant ($P < 0.0001$), with a continuous reduction of milk yield from no or very weak infected herds (assigned as BTM ELISA negative) to strong infected herds. We observed a significant reduction in the average daily milk production for strong infected herds in comparison to medium infected herds (-0.83 kg; $P < 0.0001$), low infected herds (-1.43 kg; $P < 0.0001$) and BTM ELISA negative herds (-1.62 kg; $P < 0.0001$). Moreover, the average daily milk production in medium infected herds was significantly lower compared to low infected herds (-0.64 kg; $P = 0.0001$) and BTM ELISA negative herds (-0.83 kg; $P < 0.0001$). Additionally, the *F. hepatica* infection category significantly influenced the average daily milk protein and fat yield ($P < 0.0001$). We estimated significantly lower herd protein yield daily averages in strong infected negative herds compared to medium infected herds (-0.03 kg; $P < 0.0001$), low infected herds (-0.05 kg; $P < 0.0001$) and BTM ELISA negative herds (-0.06 kg; $P < 0.0001$). A significant difference of -0.02 kg in the average daily protein yield was found between the medium and low herd infection category ($P = 0.0008$). The difference in the average daily protein yield was -0.03 kg between medium infected and BTM ELISA negative herds ($P < 0.0001$). The same effect was observed for herd fat yield with significantly lower average daily fat yields in strong infected herds compared to medium infected herds (-0.03 kg; $P < 0.0001$), low infected herds (-0.06 kg) and BTM ELISA negative herds (-0.06 kg; $P < 0.0001$). We estimated a significant reduction of -0.03 kg in the average daily fat yield in medium infected herds compared to low infected and BTM ELISA negative herds ($P < 0.0001$). The association between *F. hepatica* BTM ELISA result and herd average SCC was not statistically significant ($P = 0.664$). The full model output is shown in Table 4.

Association between* F. hepatica *BTM ELISA results and milk ß-hydroxybutyrate and acetone content

Table 3 presents the least-squares means and results of the test of significance for fixed effects (sum of squares type III) from the linear mixed model analysis for milk ketone bodies BHB and acetone. A significant association was found between *F. hepatica* infection category and milk BHB ($P = 0.002$). We identified significant higher average values for milk BHB in herds

representing a strong *F. hepatica* infection status compared to medium infected herds (+5.0 µmol/L; $P = 0.0472$), low infected herds (+6.1 µmol/L; $P = 0.0054$) and BTM ELISA negative herds (+4.4 µmol/L; $P = 0.0048$). For milk acetone, the *F. hepatica* infection category was not statistically significant in the model ($P = 0.079$). The full model output is presented in Table 5.

Table 3. Least-squares means and results of the test of significance for fixed effects (sum of squares type III) for herd average milk production parameters and milk ketone bodies ß-hydroxybutyrate (BHB) and acetone within F. hepatica herd infection categories. Different letters within column indicate significant differences ($P \leq 0.05$).

Infection category	Milk yield (kg)	Protein yield (kg)	Fat yield (kg)	SCC (cells/ml x 1,000)	BHB (µmol/L)	Acetone (µmol/L)
Strong infection	25.20 ± 0.19^{c}	0.86 ± 0.007^{c}	1.01 ± 0.007^{c}	234.82 ± 5.44^{a}	57.41 ± 1.22^{b}	62.42 ± 1.94^{a}
Medium infection	25.99 ± 0.17^{b}	0.89 ± 0.006^{b}	1.04 ± 0.006^{b}	232.15 ± 6.04^{a}	52.41 ± 1.66^{a}	58.36 ± 2.79^{a}
Low infection	26.63 ± 0.16^{a}	0.91 ± 0.006^{a}	1.07 ± 0.006^{a}	227.57 ± 5.56^{a}	51.30 ± 1.51^{a}	55.46 ± 2.53^{a}
No or very weak infection	26.82 ± 0.13^{a}	0.92 ± 0.005^{a}	1.07 ± 0.004^{a}	233.04 ± 3.85^{a}	52.98 ± 0.75^{a}	57.93 ± 1.23^{a}
P-value	<0.0001	<0.0001	<0.0001	0.664	0.002	0.079

Table 4. Fixed-effect parameter estimates with corresponding confidence limits (95% CI), *P*-values for fixed effects and covariance parameter estimates for the average herd test-day milk production parameters milk yield (kg/cow/day), protein yield (kg), fat yield (kg) and somatic cell count (SCC) from the multivariable linear mixed model analysis.

	Milk yield		**Protein yield**		**Fat yield**		**SCC**	
	Estimate (95% CI)	*P*-value	Estimate (95% CI)	*P*-value	Estimate (95% CI)	*P*-value	Estimate (95% CI)	*P*-value
Intercept	32.58 (30.85; 34.31)	$<10^{-4}$	1.07 (1.01; 1.14)	$<10^{-4}$	1.24 (1.17; 1.31)	$<10^{-4}$	107.3 (25.30; 189.25)	0.01
F. hepatica infection status		$<10^{-4}$		$<10^{-4}$		$<10^{-4}$		0.66
No or weak infection	1.63 (1.27; 1.99)	$<10^{-4}$	0.06 (0.04; 0.07)	$<10^{-4}$	0.06 (0.05; 0.07)	$<10^{-4}$	-1.78 (-12.15; 8.86)	0.74
Low infection	1.44 (1.07; 1.9)	$<10^{-4}$	0.05 (0.04; 0.06)	$<10^{-4}$	0.06 (0.04; 0.07)	$<10^{-4}$	-7.25 (-19.90; 5.40)	0.26
Medium infection	0.79 (0.48; 1.10)	$<10^{-4}$	0.03 (0.02; 0.04)	$<10^{-4}$	0.03 (0.02; 0.04)	$<10^{-4}$	-2.67 (-14.71; 9.36)	0.66
Strong infection	Baseline		Baseline		Baseline		Baseline	
Test-day date (month x year)		$<10^{-4}$		$<10^{-4}$		$<10^{-4}$		$<10^{-4}$
May 2017	0.96 (-0.63; 2.56)	0.24	-0.01 (-0.07; 0.05)	0.76	-0.01 (-0.07; 0.05)	0.77	23.03 (-55.01; 101.07)	0.56
June 2017	0.79 (-0.78; 2.37)	0.32	-0.03 (-0.09; 0.03)	0.38	-0.04 (-0.10; 0.02)	0.17	21.01 (-56.08; 98.11)	0.59
July 2017	0.20 (-1.38; 1.78)	0.80	-0.05 (-0.11; 0.01)	0.11	-0.07 (-0.12; -0.01)	0.03	43.30 (-33.90; 120.50)	0.27
August 2017	-0.19 (-1.77; 1.38)	0.81	-0.04 (-0.10; 0.02)	0.16	-0.07 (-0.13; -0.01)	0.02	35.97 (-41.09; 113.02)	0.36
September 2017	-1.29 (-2.87; 0.28)	0.12	-0.06 (-0.12; 0.00)	0.05	-0.07 (-0.13; -0.01)	0.02	25.03 (-52.03; 102.09)	0.52
October 2017	-1.98 (-3.56; -0.41)	0.01	-0.08 (-0.14; -0.02)	0.01	-0.07 (-0.13; -0.01)	0.03	21.26 (-55.82; 98.34)	0.59
November 2017	-1.92 (-3.50; -0.35)	0.02	-0.07 (-0.13; -0.01)	0.02	-0.05 (-0.11; 0.01)	0.11	23.33 (-53.87; 100.31)	0.55
December 2017	-0.64 (-2.42; 1.15)	0.48	-0.02 (-0.09; 0.05)	0.52	-0.02 (-0.09; 0.05)	0.57	-8.97 (-96.50; 78.56)	0.84
May 2018	1.01 (-0.58; 2.59)	0.21	-0.02 (-0.08; 0.04)	0.52	-0.01 (-0.07; 0.05)	0.86	25.49 (-52.07; 103.06)	0.52
June 2018	0.74 (-0.84; 2.31)	0.36	-0.04 (-0.10; 0.02)	0.23	-0.06 (-0.12; 0.002)	0.06	30.27 (-46.77; 107.32)	0.44
July 2018	-0.14 (-1.71; 1.44)	0.86	-0.07 (-0.13; -0.01)	0.02	-0.09 (-0.15; -0.03)	0.002	26.71 (-50.38; 103.80)	0.50
August 2018	-0.81 (-2.38; 0.76)	0.31	-0.08 (-0.14; -0.02)	0.01	-0.10 (-0.16; -0.04)	0.001	57.80 (-19.17; 134.78)	0.14
September 2018	-1.09 (-2.66; 0.49)	0.18	-0.06 (-0.12; 0.003)	0.06	-0.07 (-0.13; -0.01)	0.01	27.57 (-49.43; 104.58)	0.48

	Milk yield		**Protein yield**		**Fat yield**		**SCC**	
	Estimate (95% CI)	*P*-value	Estimate (95% CI)	*P*-value	Estimate (95% CI)	*P*-value	Estimate (95% CI)	*P*-value
October 2018	-1.68 (-3.26; -0.11)	0.04	-0.06 (-0.12; 0.001)	0.05	-0.06 (-0.12; 0.0003)	0.05	6.70 (-70.30; 83.71)	0.86
November 2018	-1.68 (-3.43; -0.28)	0.02	-0.06 (-0.12; 0.002)	0.06	-0.04 (-0.10; 0.02)	0.16	0.80 (-76.30; 77.89)	0.93
December 2018	Baseline		Baseline		Baseline		Baseline	
Average DIM	-0.04 (-0.046; -0.043)	$<10^{-4}$	-0.001 (-0.001; -0.001)	$<10^{-4}$	-0.001 (-0.001; -0.001)	$<10^{-4}$	0.13 (0.05; 0.21)	0.001
Average lactation number	0.57 (0.37; 0.78)	$<10^{-4}$	0.02 (0.01; 0.02)	$<10^{-4}$	0.03 (0.02; 0.04)	$<10^{-4}$	29.33 (21.57; 37.09)	$<10^{-4}$
Random effects	Estimate		Estimate		Estimate		Estimate	
Herd	16.45		0.02		0.02		6361.8	
Residual	2.65		0.004		0.004		6549.9	

Table 5. Fixed-effect parameter estimates with corresponding confidence limits (95% CI) as well as *P*-values for fixed effects and covariance parameter estimates for the milk ß-hydroxybutyrate (BHB) and milk acetone content from the multivariable linear mixed model analysis.

	Milk BHB content		**Milk acetone content**	
	Estimate (95% CI)	*P*-value	Estimate (95% CI)	*P*-value
Intercept	71.66 (62.92; 80.40)	$<10^{-4}$	84.91 (67.99; 101.83)	$<10^{-4}$
F. hepatica infection status		0.002		0.079
No or weak infection	-4.43 (-7.01; -1.84)	0.0008	-4.49 (-8.45; -0.53)	0.0263
Low infection	-6.12 (-9.71; -2.50)	0.0009	-6.96 (-12.82; -1.10)	0.0199
Medium infection	-5.00 (-8.64; -1.36)	0.0071	-4.06 (-10.20; -2.09)	0.1957
Strong infection	Baseline		Baseline	
Test-day date (month x year)				
July 2017	-11.18 (-21.07; -1.28)	0.0268	62.05 (42.40; 81.70)	$<10^{-4}$
August 2017	-9.29 (-17.60; -0.97)	0.0286	63.81 (47.51; 80.11)	$<10^{-4}$
September 2017	-10.00 (-18.25; -1.76)	0.0174	57.88 (41.73; 74.04)	$<10^{-4}$
October 2017	-6.58 (-14.84; 1.69)	0.1191	56.97 (40.77; 73.17)	$<10^{-4}$
November 2017	-5.18 (-13.41; 3.06)	0.2179	55.32 (39.19; 71.45)	$<10^{-4}$
December 2017	-5.74 (-14.17; 2.69)	0.1823	58.20 (41.67; 74.72)	$<10^{-4}$
January 2018	-3.61 (-11.85; 4.63)	0.3901	52.71 (36.57; 68.86)	$<10^{-4}$
February 2018	-0.12 (-8.57; 8.33)	0.9778	52.00 (35.42; 68.57)	$<10^{-4}$
March 2018	3.64 (-9.33; 16.62)	0.5821	40.59 (15.16; 66.02)	0.0018
July 2018	-8.16 (-17.22; 0.90)	0.0777	-16.97 (-34.69; 0.76)	0.0606
August 2018	-2.70 (-10.90; 5.51)	0.5194	-12.35 (-28.39; 3.68)	0.1310
September 2018	-16.39 (-24.57; -8.21)	$<10^{-4}$	-28.71 (-44.71; -12.71)	0.0004
October 2018	-20.90 (-29.10; 12.70)	$<10^{-4}$	-20.73 (-36.79; -4.68)	0.0114
November 2018	-14.05 (-22.21; -5.88)	0.0007	-19.80 (-35.78; -3.83)	0.0151
December 2018	-7.00 (-15.35; 1.35)	0.1001	-3.26 (-19.58; 13.07)	0.6958
January 2019	-11.64 (-19.79; -3.48)	0.0052	-10.98 (-26.94; 4.98)	0.1776
February 2019	-10.69 (-18.93; -2.46)	0.0110	-12.46 (-28.58; 3.67)	0.1300
March 2019	Baseline		Baseline	
Average DIM	-0.45 (-0.52; -0.38)	$<10^{-4}$	-1.33 (-1.48; -1.19)	$<10^{-4}$
Average lactation number	2.56 (1.91; 3.21)	$<10^{-4}$	-0.65 (-1.93; 0.63)	0.3168
Random effects	Estimate		Estimate	
Herd	277.38		413.12	
Residual	1037.73		4097.89	

Discussion

The BTM ELISA results of this study revealed moderate *F. hepatica* herd seroprevalences in the German region East Frisia, Lower Saxony, with *F. hepatica* antibodies identified in 33.1% of BTM samples in 2017 and 37.0% of BTM samples in 2018. Here, we analysed BTM samples collected between October and December, with more than 70% of samples collected in November. In this follow-up to the studies by Kuerpick et al. (2012a, 2012b), levels of seropositivity are lower than those of 48.4%, 53.9% and 53.6% reported for November 2010, 2008 and 2006, respectively. Since farmers were informed about the BTM ELISA results of these previous studies, the decrease in the present study could be the consequence of implementation of effective *F. hepatica* control measures. Nevertheless, differences in the study design (e.g., dairy factory, geographical districts within East Frisia) may have contributed to lower seroprevalences in our study compared to those reported by Kuerpick et al. (2012a, b). Noteworthy, the year 2018 was considered to be the warmest (average temperature of 10.5 °C) and fourth driest year (drought from February to November) in Germany since the beginning of weather recordings in 1881 (German Meteorological Service, 2019), and drought continued in 2019 so far (Helmholtz Centre for Environmental Research, 2019). Thus, *F. hepatica* infections in German cattle are supposed to further decrease in 2019, and potentially the following years, due to unfavourable conditions for *F. hepatica* egg development and the snail intermediate host, both requiring moist pasture conditions.

In a German geospatial mapping study, Kuerpick et al. (2013) identified the highest herd seroprevalences in the Northern German federal states Schleswig-Holstein (38.4%) and Lower Saxony (including the region East Frisia) (29.4%) in the year 2008, whereas seroprevalences were the lowest (< 6%) in the East of Germany. Regional variations in seroprevalences can be explained again by unfavourable conditions for the intermediate host, but additionally by housing conditions (high proportion of outdoor systems with grazing in the north vs. high proportion of indoor systems in the East of Germany). Olsen et al. (2015) reported herd seroprevalences of up to 30% in Denmark, based on abattoir surveillance data between the years 2011 to 2013. Arias et al. (2011) identified herd seroprevalences of up to 28% in Northern Portugal and Spain, whereas seroprevalences of up to 37% were found in dairy herds in Belgium (Bennema et al., 2009). In the UK and in Ireland, fasciolosis is a much more persistent problem with over 93% *F. hepatica* positive BTM samples in Northern Ireland (Byrne et al., 2018).

Of all *F. hepatica* positive herds in the present study, the highest proportion of herds belonged to the strong infection category (> 50% of herd infected), which is in accordance with results

reported by Byrne et al. (2018) and Kuerpick et al. (2012b). This finding reflects persistent infections in herds due to grazing on contaminated pastures as well as the persistence of antibodies of up to two years even if the infection has been cleared (Ortiz et al., 2000). In accordance, we tested 76.8% of the herds with repeated BTM measurements positive in both sampling years. Overall, fasciolosis is still an animal health problem in Germany with about one-third seropositive dairy herds. This is mainly due to insufficient treatment options in dairy cows. Triclabendazole, which is effective against adult and juvenile flukes, is licensed for dairy cows only during the dry period (with the limitation that treatment is allowed only up to 45-41 days prior to calving), while oxyclozanide, which has a withdrawal period of 4.5 days for milk, is effective against adult flukes only.

We estimated a significant negative association between positive *F. hepatica* BTM ELISA results with the milk production parameters average daily milk yield, average daily milk protein yield and average daily milk fat yield. We observed a reduction of -1.62 kg per cow per day in strongly infected herds compared to those with a negative BTM ELISA result. This finding is comparable to the findings by Mezo et al. (2011), who described a significant loss of 2 kg per cow per day based on *F. hepatica* antibody levels for individual cows. Reductions of up to 4.2 kg per cow per day in the average herd milk yield for herds representing high BTM anti-*F. hepatica* antibody levels were also observed by other authors (Charlier et al., 2007; Howell et al., 2015; Köstenberger et al., 2017). In contrast, May et al. (2019) identified no relationship between patent *F. hepatica* infections (measured by faecal egg counts [FEC]) and milk yield per cow per day based on a dataset of 1166 cows from 17 German dairy herds. In the current study, we utilized linear mixed models to estimate least-squares means for different *F. hepatica* infection categories. For the three production parameters average daily milk yield, average daily milk protein yield and average daily milk fat yield, least-squares means decreased by increasing herd infection categories (i.e., lowest values for production parameters in strongly infected herds). However, the test result revealed no significant difference between BTM ELISA negative herds with an average milk yield of 26.82 kg and low infected herds with an average of 26.63 kg milk per cow per day. Thus, a significant negative effect on milk yield was only observed as of > 20% infected cows within the herd.

The *F. hepatica* herd infection category significantly influenced milk protein and fat yield in the current study. We identified an average 0.06 kg higher milk protein and fat yield in BTM ELISA negative herds compared with strongly infected herds. Charlier et al. (2007) associated an increase in serum *F. hepatica* herd antibody levels from the 25% to the 75% quantile with a

decrease of 0.06% in the average herd milk-fat % via multivariable regression analysis. Köstenberger et al. (2017) estimated a significant decrease of 0.06 to 0.09% in milk protein and butterfat content in herds with high *F. hepatica* BTM antibody levels. Other authors did not find significant association between herd or individual *F. hepatica* infection status measured via antibody levels or FEC and milk protein or fat content (Charlier et al., 2012; Howell et al., 2015; May et al., 2019; Mezo et al., 2011). Here, we focused on milk protein and fat yield as outcome variables to account for the naturally negative dilution effect of milk yield on milk protein and fat content (Ikonen et al., 2004). A decrease in milk protein and fat yield might be a result of negative energy balance (Reist et al., 2002), probably induced by reduced feed intake and feed-conversation ratios in *F. hepatica* infected cows (Kaplan, 2001). Another explanation for decreased milk protein and fat yield addresses physiological changes in patent *F. hepatica* infected hosts due to a decrease of liver enzymes involved in energy metabolism (Baldissera et al., 2015; Phiri et al., 2007). As a further effect, *F. hepatica* induces immune suppression in its host (Molina-Hernández et al., 2015), raising the importance to study the effect of *F. hepatica* infections on further economic relevant infectious diseases in cattle (e.g., Lucena et al., 2017). Negative associations between fluke infections with bacterial infections by *Bordetella bronchiseptica*, *Mycobacterium bovis* and *Salmonella dublin* were well reported (Aitken et al., 1978; Claridge et al., 2012; Lucena et al., 2017). In this regard, we aimed to investigate the relationship between *F. hepatica* infections assessed by herd antibody levels with herd average milk SCC as an indicator for mastitis, the most important economic bacterial disease in dairy cows. We detected no significant effect of *F. hepatica* infection category on SCC in the model analysis, which is in accordance to the estimations of Howell et al. (2015) and Mezo et al. (2011) based on *F. hepatica* antibody results. Interestingly, a study based on 1166 patent infected cows (measured by FEC) confirmed that an influential effect of *F. hepatica* infections on SCC can be excluded. Nevertheless, it is possible that an effect of fasciolosis on mastitis might be more obvious when focusing on specific mastitis pathogens, since immune response during mastitis differs in dependency of bacterial group (Oviedo-Boyso et al., 2007).

This is the first study estimating the association between anti-*F. hepatica* antibody status of dairy herds and average herd milk ketone bodies BHB and acetone assessed by FTIR spectrometry from routine milk recording. Milk BHB and acetone from FTIR spectrometry are commonly used biomarkers to diagnose primary ketosis in dairy cows (Heuer et al., 2001; Santchi et al., 2016; Tatone et al., 2017), albeit phenotypic correlations between milk and blood BHB with producer recorded ketosis data are low (Belay et al., 2017; Koeck et al., 2014). One

explanation for low correlations addresses the subjective diagnosis of ketosis, resulting in inconsistent recording between herds and employees within herds (Zwald et al., 2004). As a further justification for the low correlations between milk or blood BHB and producer recorded ketosis diagnoses, we hypothesized an increase in herd milk or blood ketone bodies due to *F. hepatica* infections, especially in the absence of clinical signs for primary ketosis. Only one research study addressed the relationship between patent *F. hepatica* infections in 203 Polish dairy cows and serum glucose and BHB (Kowalczyk et al., 2018), but no significant differences in the values between chronically infected and non-infected cows were identified. In contrast, *F. hepatica* infections affected serum glucose levels and serum ß-hydroxybutyrate in sheep (Berry and Dargie, 1976; Ferre et al., 1994; Kozat and Denizhan, 2010; Phiri et al., 2007; Yüksek et al., 2013). A stronger effect of fluke infections on serum or milk ketone bodies is conceivable in sheep, since cattle are more resistant to and able to overcome liver fluke (re-)infections (e.g., due to calcification of bile ducts) (Hillyer et al., 1996). Here, we estimated a significant negative association between herd fluke antibody level and the average herd milk BHB with significantly higher average milk BHB values in herds representing a strong infection status compared to the other three infection categories. However, the effect of *F. hepatica* herd infection category on milk acetone content was not significant, albeit strongly infected herds showed a tendency for higher acetone values compared with BTM ELISA negative herds. Hence, beside primary ketosis, *F. hepatica* infections should be considered as a potential further risk factor for increased milk BHB and acetone values in dairy herds.

Heuer et al. (2001) and Chandler et al. (2018) estimated mean milk acetone values in a range between 70-1000 µmol/L by FTIR spectrometry, which is higher than the mean acetone value of 40 µmol/L in our dataset. The mean milk BHB of 48.6 µmol/L in our test-day samples was in the same range of those identified by Chandler et al. (2018) with a mean of 73 µmol/L milk BHB in Holstein cows predicted by FTIR spectrometry. Belay et al. (2017) reported higher mean BHB values of 1200 µmol/L and a broader range of -4050-6317 µmol/L in Norwegian Red cows between DIM 11 and 60. Validation studies aiming on the application of milk ketone bodies from FOSS ketosis screening calibration predicted a high accuracy and sensitivity for the use of FTIR spectrometry milk BHB to assess ketosis on a herd-level (Chandler et al., 2018; Renaud et al., 2019). In current ketosis monitoring programs developed on the basis of FOSS ketosis screening calibrations, the prediction of within-herd prevalences for ketosis is based on the proportion of milk BHB values ≥ 0.15 mmol/l at first test-day record within the first 30 or 60 DIM (e.g., Ketodetect, CLASEL, France; ketosis screening by CRV and Qlip, the

Netherlands; Ketolab, Valacta, Kanada; Schwarz et al., 2015). Hence, we focused in our analysis on cow records for milk BHB and acetone values between DIM 5 and 60. However, when all records as of DIM 5 for BHB and acetone were considered in the analysis, the relationship between *F. hepatica* infection category and average milk BHB remained significant (data not shown). Unfortunately, it was not possible to include ketosis diagnosis data recorded by producers or veterinarians as a risk factor for increased milk BHB and acetone values in our analysis. Moreover, limitations in the model analysis are given by the application of two indirect measured parameters: first, the herd antibody level as an indicator for *F. hepatica* infections, which does not allow a differentiation between the current infection status and a previous exposure (Mezo et al., 2011), and second, milk ketone bodies predicted by FOSS ketosis screening calibrations with only moderate correlation coefficients between 0.40 to 0.85 with chemically determined milk BHB or serum BHB (Chandler et al., 2018; de Roos et al., 2007; Renaud et al., 2019). Nevertheless, examining a large number of herds for patent *F. hepatica* infections and serum or plasma BHB and acetone levels of individual cows is time-consuming and costly. Further studies are needed to investigate the relationship between *F. hepatica* infections and their effect on cow metabolic health and energy deficiency. We recommend to consider *F. hepatica* infections, beside primary ketosis, as one risk factor for increased milk BHB in dairy herds.

Conclusions

This study of BTM *F. hepatica* antibody levels revealed seroprevalences of 33.1% in 2017 and 37.0% in 2018 in dairy herds in the northern German region East Frisia, which are substantially lower compared to prior seroprevalence studies conducted in 2006, 2008 and 2010 in East Frisia, showing seroprevalences of more than 45%. We identified a significant association between *F. hepatica* infection category and milk production parameters milk yield, milk protein yield and milk fat yield. Losses of -1.62 kg in the average daily milk yield and -0.06 kg in the average daily protein and fat yield per cow were estimated in strongly infected herds compared to BTM ELISA negative herds. The relationship between herd *F. hepatica* infection category and herd average SCC was not significant. We estimated significant higher average BHB values in strongly infected herds compared to the other three infection categories, albeit an effect of *F. hepatica* herd antibody levels on herd average acetone values was not observed. Hence, a high proportion of cows infected with *F. hepatica* within a herd might contribute to an increase in herd milk BHB predicted from FTIR spectrometry.

References

Aitken MM, Jones GW, Hall GA, Hughes DL, Collis KA. 1978. Effects of experimental Salmonella Dublin infection in cattle given *Fasciola hepatica* thirteen weeks previously. Journal of Comparative Pathology 88:75-84.

Arias M, Lomba C, Dacal V. Vázquez L, Pedreira J, Francisco I, Piñeiro P, Cazapal-Monteiro C, Suárez JL, Díez-Bãnos P, Morrondo P, Sánchez-Andrade R, Paz-Silva A. 2011. Prevalence of mixed trematode infections in an abattoir receiving cattle from northern Portugal and north-west Spain. Veterinary Record 186:408-412.

Baird GD. 1982. Primary ketosis in the high-producing dairy cow: Clinical and subclinical disorders, treatment, prevention, and outlook. Journal of Dairy Science 65:1-10.

Baldissera MD, Bottari NB, Mendes RE, Schwertz CI, Lucca NJ, Dalenogare D, Bochi GV, Moresco RN, Morsch VM, Schetinger MRC, Rech VC, Jaques JA, Da Silva AS. 2015. Activity of cholinesterases, pyruvate kinase and adenosine deaminase in rats experimentally infected by *Fasciola hepatica*: Influences of these enzymes on inflammatory response and pathological findings. Pathology, Research and Practice 211:871-876.

Belay TK, Svendsen M, Kowalski ZM, Ådnøy T. 2017. Genetic parameters of blood ß-hydroxybutyrate predicted from milk infrared spectra and clinical ketosis, and their associations with milk production traits in Norwegian Red cows. Journal of Dairy Science 100:6298-6311.

Bennema S, Vercruysse J, Clearebout E, Schnieder T, Strube C, Ducheyne E, Hendrickx G, Charlier J. 2009. The use of bulk-tank milk elisas to assess the spatial distribution of *Fasciola hepatica*, *Ostertagia ostertagi* and *Dictyocaulus viviparus* in dairy cattle in flanders (Belgium). Veterinary Parasitology 165:51-57.

Berry CI, Dargie JD. 1976. The role of host nutrition in the pathogenesis of ovine fascioliasis. Veterinary Parasitology 2:317-322.

Brady MT, O'Neill SM, Dalton JP, Mills KHG. 1999. *Fasciola hepatica* suppresses a protective Th1 response against *Bordetella pertussis*. Infection and Immunity 67:5372-5378.

Byrne AW, Graham J, McConville J, Milne G, McDowell S, Hanna REB. 2018. Seasonal variation of *Fasciola hepatica* antibodies in dairy herds in Northern Ireland measured by bulk tank milk ELISA. Parasitology Research 117:2725-2733.

Chandler TL, Pralle RS, Dórea JRR, Poock SE, Oetzel GR, Fourdraine RH, White HM. 2018. Predicting hyperketonemia by logistic and linear regression using test-day milk and performance variables in early-lactation Holstein and Jersey cows. Journal of Dairy Science 101:2476-2491.

Charlier J, Duchateau L, Clearebout E, Williams D, Vercruysse J. 2007. Associations between anti-*Fasciola hepatica* antibody levels in bulk-tank milk samples and production parameters in dairy herds. Preventive Veterinary Medicine 78:57-66.

Charlier J, De Cat A, Forbes A, Vercruysse J. 2009. Measurement of antibodies to gastrointestinal nematodes and liver fluke in meat juice of beef cattle and associations with carcass parameters. Veterinary Parasitology 166:235-240.

Charlier J, Hostens M, Jacobs J, Van Ranst B, Duchateau L, Vercruysse J. 2012. Integrating fasciolosis control in the dry cow management: the effect of closantel treatment on milk production. PloS One 7:e43216.

Claridge J, Diggle P, McCann CM, Mulcahy G, Flynn R, McNair J, Strain S, Welsh M, Baylis M, Williams DJL. 2012. *Fasciola hepatica* is associated with the failure to detect bovine tuberculosis in dairy cattle. Nature Communications 3:853.

De Roos APW, van den Bijgaart HJCM, Hørlyk J, de Jong G. 2007. Screening for subclinical ketosis in dairy cattle by Fourier Transform Infrared Spectrometry. Journal of Dairy Science 90:1761-1766.

Ferre I, Barrio JP, Gonzalez-Gallego J, Rojo-Vazquez FA. 1994. Appetite depression in sheep experimentally infected with *Fasciola hepatica*. Veterinary Parasitology 55:71-79.

Fox NJ, White PCL, McClean CJ, Marion G, Evans A. 2011. Predicting impacts of climate change on *Fasciola hepatica* risk. PloS One 6:e16126.

Gaddis KLP, Megonigal Jr. JH, Clay JS, Wolfe CW. 2018. Genome-wide association study for ketosis in US Jerseys using producer-recorded data. Journal of Dairy Science 101:413-424.

German Meteorological Service, 2019. https://www.dwd.de/DE/leistungen/besondereereignisse/temperatur/20190102_waermstes_jahr_in_deutschland_2018.pdfblob=publicationFile&v=2. Accessed 09 October 2019.

Helmholtz Centre for Environmental Research, 2019. https://www.ufz.de/index.php?de=37937. Accessed 09 October 2019.

Heuer C, Luinge HJ, Lutz ETG, Schukken YH, van der Maas JH, Wilmink H, Noordhuizen JPTM. 2001. Determination of acetone in cow milk by Fourier Transform Infrared Spectrometry for the detection of subclinical ketosis. Journal of Dairy Science 84:575-582.

Hillyer GV, Soler de Galanes M, Buchón P, Bjorland J. 1996. Herd evaluation by enzyme-linked immunosorbent assay for the determination of *Fasciola hepatica* infection in sheep and cattle from Altiplano of Bolivia. Veterinary Parasitology 61:211-220.

Höglund J, Dahlstrom F, Engstrom A, Hessle A, Jakubek E-B, Schnieder T, Strube C, Sollenberg S. 2010. Antibodies to major pasture borne helminth infections in bulk-milk samples from organic and nearby conventional dairy herds in south-central Sweden. Veterinary Parasitology 171:293-299.

Howell A, Baylis M, Smith R, Pinchbeck G, Williams D. 2015. Epidemiology and impact of *Fasciola hepatica* exposure in high-yielding dairy herds. Preventive Veterinary Medicine 121:41-48.

Ikonen T, Morri S, Tyrisevä A-M, Ruottinen O, Ojala M. 2004. Genetic and phenotypic correlations between milk coagulation properties, milk production traits, somatic cell count, casein content, and pH of milk. Journal of Dairy Science 87:458-467.

Kaplan RM. 2001. *Fasciola hepatica*: a review of the economic impact in cattle and considerations for control. Veterinary Therapeutics 2:40-50.

Koeck A, Jamrozik J, Schenkel FS, Moore RK, Lefebvre DM, Kelton DF, Miglior F. 2014. Genetic analysis of milk ß-hydroxybutyrate and its association with fat-to-protein ratio, body condition score, clinical ketosis, and displaced abomasums in early first lactation of Canadian Holsteins. Journal of Dairy Science 97:7286-7292.

Kowalczyk SJ, Czopowicz M, Weber CN, Müller E, Kaba J. 2018. Accuracy of a diagnostic model based on serum biochemical parameters in detecting cows at an increased risk of chronic fasciolosis. Veterinary Parasitology 254:15-20.

Kozat S, Denizhan V. 2010. Glucose, lipid, and lipoprotein levels in sheep naturally infected with *Fasciola hepatica*. Journal of Parsitology 96:657-659.

Köstenberger K, Tichy A, Bauer K, Pless P, Wittek T. 2017. Associations between fasciolosis and milk production, and the impact of anthelmintic treatment in dairy herds. Parasitology Research 116:1981-1987.

Kuerpick B, Schnieder T, Strube C. 2012a. Seasonal pattern of *Fasciola hepatica* antibodies in dairy herds in Northern Germany. Parasitology Research 111:1085-1092.

Kuerpick B, Fiedor C, von Samson-Himmelstjerna G, Schnieder T, Strube C. 2012b. Bulk milk-estimated seroprevalence of *Fasciola hepatica* in dairy herds and collecting of risk factor data in East Frisia, Northern Germany. Berliner und Münchener Tierärztliche Wochenschrift 125:345-350.

Kuerpick B, Conraths FJ, Staubach C, Fröhlich A, Schnieder T, Strube C. 2013. Seroprevalence and GIS-supported risk factor analysis of *Fasciola hepatica* infections in dairy herds in Germany. Parasitology 140:1051-1060.

Lucena, A.N., Cuartero, L.G., Mulcahy, G., Zintl, A., 2017. The immunoregulatory effects of co-infection with *Fasciola hepatica*: From bovine tuberculosis to Johne's disease. Veterinary Journal 222:9-16.

May K, Brügemann K, König S, Strube C. 2019. Patent infections with *Fasciola hepatica* and paramphistomes (*Calicophoron daubneyi*) in dairy cows and association of fasciolosis with individual milk production and fertility parameters. Veterinary Parasitology 267:32-41.

Mehmood K, Zhang H, Sabir AJ, Abbas RZ, Ijaz M, Durrani AZ, Saleem MH, Rehman MU, Iqbal MK, Wang Y, Ahmad HI, Abbas T, Hussain R, Ghori MT, Ali S, Khan AU, Li J. 2017. A review on epidemiology, global prevalence and economic losses of fasciolosis in ruminants. Microbial Pathogenesis 109:253-262.

Mezo M, Gonzalez-Warleta M, Antonio Castro-Hermida J, Muino L, Ubeira FM. 2011. Association between anti-*F. hepatica* antibody levels in milk and production losses in dairy cows. Veterinary Parasitology 180:237-242.

Molina-Hernández V, Mulcahy G, Pérez J, Martínez-Moreno Á, Donnelly S, O'Neill SM, Dalton JP, Cwiklinski K. 2015. *Fasciola hepatica* vaccine: We may not be there yet but we're on the right road. Veterinary Parasitology 208:101-111.

Olsen A, Frankena K, Toft N, Thamsborg SM, Enemark HL, Halasa T. 2015. Prevalence, risk factors and spatial analysis of liver fluke infections in Danish cattle herds. Parasites & Vectors 8:160.

Ortiz PL, Claxton JR, Clarkson MJ, McGarry J, Williams DJL. 2000. The specificity of antibody responses in cattle naturally exposed to *Fasciola hepatica*. Veterinary Parasitology 93:121-134.

Oviedo-Boyso J, Valdez-Alarcón JJ, Cajero-Juárez M, Ochoa-Zarzosa A, López-Meza JE, Bravo-Patiño A, Baizabal-Aguirre VM. 2007. Innate immune response of bovine mammary gland to pathogenic bacteria responsible for mastitis. Journal of Infection 54:399-409.

Phiri IK, Phiri AM, Harrison IJS. 2007. The serum glucose and ß-hydroxybutyrate levels in sheep with experimental *Fasciola hepatica* and *Fasciola gigantica* infection. Veterinary Parasitology 143:287-293.

Pineda A, Cardoso FC. 2015. Technical note: Validation of a handheld meter for measuring ß-hydroxybutyrate concentrations in plasma and serum from dairy cows. Journal of Dairy Science 98:8818-8824.

Reist M, Erdin D, von Euw D, Tschuemperlin K, Leuenberger H, Chilliard Y, Hammon HM, Morel C, Philipona C, Zbinden Y, Kuenzi N, Blum JW. 2002. Estimation of energy balance at the individual and herd level using blood and milk traits in high-yielding dairy cows. Journal of Dairy Science 85:3314-3327.

Renaud DL, Kelton DF, Duffield TF. 2019. Short communication: Validation of a test-day milk test for ß-hydroxybutyrate for identifying cows with hyperketonemia. Journal of Dairy Science 102:1589-1593.

SAS Institute. Inc. 2013. Version 9.4. SAS Institute Inc., Cary, NC.

Schwarz D, Lefebvre DM, van den Bijgaart H, Davière J-B, van der Linde R, Kold-Christensen S. 2015. Global experience on ketosis screening by FTIR technology. Proc. Technical Meeting of the International Committee for Animal Recording (ICAR), Krakow, Poland, ICAR, Rime, Italy (2015), pp. 5-10, ICAR technical series no. 19.

Schweizer G, Braun U, Deplazes P, Torgerson PR. 2005. Estimating the financial losses due to bovine fasciolosis in Switzerland. Veterinary Records 157:188-193.

Tremblay M, Kammer M, Lange H, Plattner S, Baumgartner C, Stegeman JA, Duda J, Mansfeld R, Döpfer D. 2018. Identifying poor metabolic adaptation during early lactation in dairy cows using cluster analysis. Journal of Dairy Science 101:7311-7321.

Tatone EH, Duffield TF, LeBlanc SJ, DeVries TJ, Gordon JL. 2017. Investigating the within-herd prevalence and risk factors for ketosis in dairy cattle in Ontario as diagnosed by the test-day concentration of ß-hydroxybutyrate in milk. Journal of Dairy Science 100:1308-1318.

Yüksek N, Altuğ N, Denizhan V, Ceylan E, Ağaoğlu Z. 2013. Enhancement of the glucose metabolism and the reverse cholesterol transport by a peroxisome proliferator receptor α (PPARα) agonist included in the fasciolosis treatment in naturally infested sheep. Revue de Médecine Vétérinaire 164:163-172.

Zwald NR, Weigel KA, Chang YM, Welper RD, Clay JS. 2004. Genetic selection for health traits using producer-recorded data. I. Incidence rates, heritability estimates, and sire breeding values. Journal of Dairy Science 87:4287-4294.

KAPITEL 6

Diskussion

6.1 Genetisch-statistische Modellierungen zur Endoparasitenresistenz

Klassischerweise werden in der Milchviehzüchtung Genotyp-Umwelt-Interaktionen (GUI) untersucht, um Unterschiede in Resistenz, Toleranz und Widerstandsfähigkeit zu ermitteln. Genotyp-Umwelt-Interaktion meint, dass verschiedene Genotypen unterschiedlich auf Änderungen in der Umwelt reagieren. Random-Regressions-Modelle (RRM) werden angewandt, um Schwankungen im Leistungsniveau in Abhängigkeit von Änderungen in der Haltungsumwelt zu schätzen (de Jong und Bijma, 2002). Dabei werden Reaktionsnormen genutzt, wobei das Intercept der Reaktionsnorm das Produktionsniveau und die Slope die Umweltsensitivität anzeigen (Lynch und Walsh, 1998). Verschiedenste Parameter wie z.B. das Herdenproduktionsniveau oder Temperatur-Luftfeuchte-Index werden dabei zur Beschreibung der Umwelt genutzt (Kolmodin et al., 2002; Brügemann et al., 2011). Weiterhin werden Parameter wie die Erregerbelastung der Umgebung (Rashidi, 2016) oder die durchschnittliche Herdenzellzahl als Indikator von Euterinfektionen (Streit et al., 2015) verwendet, um genetische Unterschiede einer Toleranz und Widerstandsfähigkeit in Abhängigkeit von Parametern einer Erkrankung zu schätzen. Twomey et al. (2018a) schätzten genetische (Ko)Varianzkomponenten für Milchleistungs- und Fruchtbarkeitsmerkmale in Abghängigkeit der *F. hepatica*-Prävalenz in irischen Milch- und Fleischrindern mittels RRM. Die Autoren konnten dabei GUI für Fruchtbarkeitsmerkmale, nicht hingegen für Milchproduktionsmerkmale nachweisen.

Im Rahmen der vorliegenden These wurden RRM genutzt, um genetische Korrelationen zwischen Endoparasitenmerkmalen und Milchleistungsmerkmalen über den Laktationsverlauf hinweg zu schätzen (Kapitel 2). Als Basisfunktion zur Modellierung der Laktationsverlaufes wurden Legendre Polynome verwendet. Legendre Polynome haben den Nachteil, dass sie weniger robust gegenüber einer niedrigen Anzahl an Beobachtungen sind (Misztal et al., 2000; Nobre et al., 2003). Dies spiegelte sich in den eigenen Datenanalysen wider, welche hohe genetische Korrelationen und Standardfehler zum Ende der Laktation aufwiesen (May et al., 2017). Meyer et al. (2005) und Bohmanova et al. (2008) konnten zeigen, dass sich die

Verwendung sogenannter „kubischer oder linearer Splines“ positiv auf die Schätzgenauigkeit und Standardfehler von (Ko)Varianzkomponenten auswirkt. In den linear gemischten Modellen (Kapitel 2, 3 und 4) zur Modellierung fixer Effekte auf die Endoparasitenmerkale wurde das Laktationsstadium nach Huth (1995) als Einflussfaktor für endoparasitäre Infektionen modelliert. Michel et al. (1979) und Perri et al. (2011) wiesen in Studien beim Rind Unterschiede in der Anfälligkeit für parasitäre Infektionen im Laktationsverlauf und für verschiedene Altersklassen nach. Im Rahmen dieser These wurden verschiedenste Modellierungen für die fixen Effekte Laktationsstadium und Laktationsnummer zur Schätzung quantitativ-genetischer Parameter von Endoparasitenmerkmalen in Kapitel 2 sowie für die GWAS-Modelle in Kapitel 3 getestet. Die in Kapitel 2, 3 und 4 verwendeten fixen Effekte zeigten dabei insgesamt die beste Modellgüte für alle drei Erregermerkmale. Dennoch zeigten sich in genomweiten Assoziationsstudien Unterschiede in der Identifizierung von SNPs bei unterschiedlichen Modellierungen des Laktationsverlaufs (z.B. Laktationsverlauf in Stadien als fixen Effekt oder Laktationstage als Kovariable) (Ergebnisse nicht dargestellt). Weiterhin kann vermutet werden, dass Merkmale verschiedener Erregerspezies verschiedene Modellierungen für fixe und zufällige Effekte erfordern, was einer weiteren Erforschung in zukünftigen Studien bedarf.

6.2 Phänotypisierung und Merkmalsverteilung

Die akkurate Schätzung von (Ko)varianzkomponenten für Gesundheitsmerkmale erfordert eine möglichst präzise Phänotypisierung. Neuenschwander et al. (2012) schätzten Heritabilitäten zwischen 0,02 und 0,21 für binär definierte Gesundheitsdaten in kanadischen Milchviehherden, welche von Landwirten erfasst wurden. Die höchste Heritabilität zeigte sich in diesen Studien in allen Rechenläufen für das Merkmal „linksseitige Labmagenverlagerung“, welches ausschließlich als binäres Merkmal erfasst werden kann und daher mit hoher Genauigkeit von den Landwirten dokumentiert wurde. Andere Erkrankungen weisen eine stärkere Heterogenität hinsichtlich der verschiedenen Krankheitsstadien auf und wurden daher mit einer schlechteren Genauigkeit erfasst, was sich nachteilig auf die Schätzung quantitativ-genetischer Parameter auswirkte. Schöpke et al. (2015) schätzten Heritabilitäten von 0,19 bis 0,52 für die infektiöse bakterielle Klauenerkrankung Mortellaro bzw. Dermatitis digitalis (DD) unter Verwendung eines detaillierten Klassifizierungsschemas, welches alle chronischen Stadien und Zwischenstadien der DD abdeckt (M-scoring system). Für weniger detaillierte DD-Stadien lagen die Heritabilitäten basierend auf linearen Modellen, Schwellenmodellen oder RRM

zwischen 0,05 und 0,14 (Gernand and König, 2014; Häggman und Juga, 2013; König et al., 2008). Die Ergebnisse der quantitativ-genetischen Studien sowie die Einführung einer sehr genauen Erfassung von DD Phänotypen in der Praxis ermöglichten die Entwicklung eines eigenen genomischen Zuchtwertes für Mortellaro (*DD control*) (https://www.vit.de/fileadmin/DE/Zuchtwertschaetzung/Zws_Bes_deu.pdf). Der Landwirt hat damit die Möglichkeit zur gezielten Auswahl von Bullen mit hohen Zuchtwerten für *DD control*, um das Auftreten von Mortellaro-Fällen in der Herde zu reduzieren. Die Zucht auf eine verbesserte Resistenz gegen infektiöse Erkrankungen lässt sich daher besonders gut für diejenigen Erkrankungen realisieren, welche ohne einen direkten Erregernachweis mittels klinischer Diagnostik eindeutig erfasst werden können.

Eine phänotypische Erfassung endoparasitärer Infektionen ist durch den Landwirt in praxi nicht möglich, da patente Infektionen in den seltensten Fällen zu äußeren klinischen Veränderungen bei der Milchkuh führen und sich allenfalls subklinisch (z.B. Rückgang der Milchleistung) äußern. Weiterhin besteht für komplexe Merkmale wie Endoparasitenresistenz neben dem Problem der exakten Phänotypisierung das Problem der Merkmalsverteilung in genetisch-statistischen Analysen, da Merkmale endoparasitärer Infektionen in der Regel nicht normalverteilt sind. Eine nicht normale Verteilung der Daten zeigte sich auch für die drei Endoparasitenmerkmale in der vorliegenden Arbeit. Insbesondere für GIN- und *D. viviparus*-Infektionen sind bei adulten Kühen aufgrund einer erworbenen Immunität nach Erstinfektion niedrigere Ei- und Larvenausscheidungen zu erwarten (May et al., 2017; Ravinet et al., 2014). Häufig hat man es daher bei endoparasitären Daten mit einer rechtschiefen Merkmalsverteilung zu tun, welche eine große Anzahl nicht-infizierter Tiere beinhaltet (Nødtvedt et al., 2002; Silva et al., 2012). Studien, welche den Effekt einer Normalverteilung komplexer Merkmale auf die Schätzung quantitativ-genetischer Parameter beleuchten, liegen derzeit nur unzureichend vor. Browne et al. (1990) und Silva et al. (2012) konnten für endoparasitäre Merkmale zeigen, dass das Fehlen einer Normalverteilung in einer verringerten Schätzgenauigkeit quantitativ-genetischer Parameter resultiert. Für Endoparasitenmerkmale werden daher verschiedenste Transformationen in quantitativ-genetischen Studien angewandt, um eine Normalverteilung der Daten zu erzielen (Nødtvedt et al., 2002). Insbesondere die Transformationen $y = \ln (FEC + 1)$ (Togersen et al., 2005) und $y = \ln (FEC + 100)$ (Morris et al. 2003) sind in der Literatur beschrieben. Silva et al. (2012) verwendeten die Box-Cox Transformation, um eine Normalverteilung des Endoparasitenmerkmals FEC zu erzielen. Die Autoren schätzten eine

Heritabilität von 0,5 für die mittels Box-Cox Transformation normalverteilten Daten, wohingegen eine Heritabilität von 0,4 für die logarithmierten Daten und eine Heritabilität von 0,21 für die untransformierten Daten geschätzt wurde. Die Box-Cox Transformation endoparasitärer, zuvor nicht-normalverteilter Daten führte somit nachweislich zu einem Anstieg der Heritabiltität. Allerdings verweisen die Autoren darauf, dass die Box-Cox Transformation komplexer Merkmale wie Endoparasitenresistenz nicht mit absoluter Zuverlässigkeit in einer Normalverteilung der Daten resultiert. Die im Rahmen dieser These geschätzten Heritabilitäten für Merkmale von GIN- bzw. *F. hepatica*-Infektionen (Kapitel 2) basieren auf untransformierten Merkmalen, da auch durch die Anwendung verschiedenster Transformationen keine Normalverteilung erzielt werden konnte. Auch die Box-Cox Transformation resultierte nicht in einer Normalverteilung der Daten. In einem weiteren Schritt verglichen May et al. (2017) Heritabilitäten für nicht normalverteilte untransformierte, logarithmierte (ln (FEC + 100)) und radizierte Merkmalsdefinitionen endoparasitärer Infektionen. Die Autoren schätzten eine höhere Heritabilität für das logarithmierte und radizierte Merkmal „*F. hepatica*-Eiausscheidung“ im Vergleich zu den nicht transformierten Daten, wohingegen sich für die anderen Endoparasitenmerkmale nach Transformation keine Änderungen in den Heritabilitäten ergaben. Zudem zeigte sich in den genetisch-statistischen Modellen durchgehend eine bessere Modellgüte für die untransformierten Rohdaten der Endoparasitenmerkmale. Die Heritabilitäten für die Endoparasitenmerkmale in dieser Studie wurden mit der AI-REML-Methode geschätzt, welche als robust gegenüber verzerrten Merkmalsverteilungen zur Varianzkomponentenschätzung gilt (Banks et al., 1985; Rubio-Aparicio et al., 2018). Auch erfolgte die Schätzung der Heritabilitäten für Endoparasitenmerkmale in dieser These auf Basis wiederholter Beobachtungen, was die Schätzgenauigkeit im Vergleich zu einmaligen Messungen erhöht. Wiederholte Messungen können ein Grund für die niedrigeren Heritabilitäten des Merkmals FEC-GIN in dieser These im Vergleich zu den höheren Heritabilitäten anderer Studien auf Basis einmaliger Beobachtungen sein, wie dies bei Silva et al. (2012) diskutiert wurde. Für die GWAS in Kapitel 3 wurden die Endoparasitenmerkmale in einem ersten Modell auf fixe Effekte vorkorrigiert und die daraus resultierenden und normalverteilten Residuen als Merkmal in der GWAS verwendet.

6.3 Genomweite Assoziationsstudien für Endoparasitenmerkmale

In der vorliegenden Arbeit wurden die Merkmale Eiausscheidung von GIN und *F. hepatica* bzw. Larvenausscheidung von *D. viviparus* als Merkmale patenter Infektionen bei Milchkühen

genutzt, um genetische Variationen zu identifizieren, die mit einer Endoparasitenresistenz assoziiert sind. Für das Merkmal rFEC-GIN lag die Mehrzahl der assoziierten Marker auf BTA 2, wohingegen Kandidatengene auf BTA 4, 5, 18, 22 und 24 identifiziert wurden. Twomey et al. (2019) nutzten *O. ostertagi*-Antikörpertests als Resistenzmerkmal in irischen Fleisch- und Milchrindern und identifizierten SNPs auf BTA 3, 4, 12, 13, 14, 21 und 23. Coppieters et al. (2009) beschrieben QTL über dem genomweiten Signifikanzniveau auf BTA 9 und 19 sowie weitere QTL auf BTA 11, 14, 21, 24, 25 und 27 für das Merkmal FEC-GIN bei HF-Kühen. Dabei bezogen die Autoren Beobachtungen von Kühen zwischen der 2. und 6. Laktation in die Analysen ein, wohingegen die GWAS der vorliegenden Arbeit auf Daten genotypisierter Kühe zwischen der 1. und. 11. Laktation basiert. Es ist davon auszugehen, dass die Vorselektion bestimmter Tiergruppen oder Individuen auf Basis des Phänotyps ebenfalls einen Einfluss auf die identifizierten Varianten in der GWAS hat. Die GWAS in Kapitel 3 basiert auf einem selektiven Genotypisierungsansatz, bei welchem die DSN Kühe auf Basis der Endoparasitenspezies mit der höchsten Prävalenz (= GIN; Prävalenz: 23,0 bis 40,0 %) im Gesamtdatensatz der 1166 Kühe ausgewählt wurden, mit der Hälfte infizierter und der Hälfte nicht-infizierter Tiere basierend auf wiederholten Beobachtungen. Für die Endoparasitenspezies *D. viviparus* und *F. hepatica* waren die Prävalenzen im Gesamtdatensatz sowie im Datensatz der genotypisierten Tiere deutlich niedriger (3,4 bzw. 10,0 %). Damit besteht die Gefahr, dass die wenigen mit *D. viviparus* oder *F. hepatica* infizierten Tiere sich für bestimmte Loci nicht stark voneinander unterscheiden, was wiederum das Risiko falsch-negativer Assoziationen für die beiden Endoparasitenmerkmale FEC-FH und FLC-DV in der GWAS erhöht.

Für das Merkmal *F. hepatica*-Eiausscheidung lag die höchste Anzahl an Assoziationen auf BTA 7, wohingegen nur ein Kandidatengen, das Activated Cell Adhesion Molecule (*ALCAM*) Gen auf BTA 1, gefunden wurde. Twomey et al. (2019) identifizierten genomische Regionen auf BTA 1, 8, 11, 16, 17 und 18, die mit dem Merkmal „Leberschädigung durch *F. hepatica*“ in Verbindung gebracht werden konnten. In der Studie von Twomey et al. (2019) wurde zudem ein QTL auf BTA 11 sowohl mit dem Merkmal „Leberschädigung durch *F. hepatica*“ als auch mit dem Merkmal „*F. hepatica*-Antikörpertiter“ assoziiert. Wie die vorliegende These nutzten Twomey et al. (2019) Infektionsmerkmale natürlich infizierter Rinder. Unter natürlichen Bedingungen im Feld unterliegen Endoparasiteninfektionen beim Rind einer Vielzahl an Umweltfaktoren (z.B. Infektionsdruck auf der Weide, Unterschiede im Weidegang), was die

Power zur Identifizierung assoziierter SNPs und die Schätzung von SNP-Effekt Korrelationen beeinflussen kann (Bishop und Woolliams, 2010). Twomey et al. (2019) identifizierten Kandidatengene für Endoparasitenresistenz in einem Datensatz, welcher mehr als zehn verschiedene Rassen beinhaltete. Purfield et al. (2019) führten genomweite Assoziationsstudien für Schlachtkörpermerkmale in einem Datensatz sechs verschiedener Rinderrassen durch. Unter Einbezug aller Rassen in der GWAS identifizierten die Autoren signifikante Assoziationen, welche bei getrennter Analyse der Rassen in der GWAS nicht gefunden werden konnten. Die Autoren grenzten mit diesem Vergleich rassespezifische Varianten für Schlachtkörpermerkmale von merkmalspezifischen Varianten für Schlachtkörpermerkmale ab. Bisherige genomische Studien für das komplexe Merkmal Endoparasitenresistenz sind bis dato nur innerhalb von Rassen oder unter Einbezug verschiedener Rassen durchgeführt worden, was eine exakte Abgrenzung zwischen merkmalsspezifischen und rassespezifischen Varianten erschwert. Für zukünftige Studien zur Endoparasitenresistenz wäre es daher ein interessanter Ansatz, genomweite Assoziationen basierend auf dem gleichen Versuchsdesign innerhalb verschiedener Rassen zu schätzen und zu vergleichen (within-breed associations) und diese mit identifizierten SNPs unter Einbezug mehrerer Rassen zu vergleichen (across-breed associations). Wie in Kapitel 1 und 2 diskutiert, ist die Erfassung einer hohen Anzahl an Phänotypen für Endoparasiteninfektionen in praxi aus zeitlichen und finanziellen Gründen oft nicht realisierbar, was eine Durchführung derartiger Analysen erschwert. Kapitel 3 dieser These fokussierte explizit auf die Identifizierung genomweiter Asszіationen für Endoparasitenmerkmale in der Rasse DSN mit dem Ziel, rassespezifische Assoziationen zu identifizieren, welche zukünftig in einer speziell für das DSN entwickelten genomischen Zuchtwertschätzung Berücksichtigung finden können.

In der vorliegenden These wurden genomweite Assoziationen für alle drei Endoparasitenmerkmale getrennt voneinander analysiert. Wie in Kapitel 1 erläutert handelt es sich bei GIN-Infektionen in der Regel um Mischinfektionen mit verschiedenen Nematodenspezies (z.B. *Ostertagia ostertagi* und *Strongyloides papillosus*), für welche sich die Immunantwort im Wirt jedoch unterscheidet (Foster und Elsheikka, 2012). Daher ist anzunehmen, dass bei differenzierter Betrachtung einzelner GIN-Spezies in einer GWAS Parasitenspezies-spezifische genetische Varianten und SNP-Effekte exakter geschätzt werden können, wie dies bereits für andere infektiöse Erreger gezeigt wurde (Mahmoud, 2019). In der Mikrobiomforschung kommen bereits seit einigen Jahren neue Modellierungsansätze wie die

Mikrobiome-GWAS (Hua et al., 2015) und MiRKAT (Zhao et al., 2015) zur Anwendung, um genetische Faktoren auf die Zusammensetzung des Mikrobioms zu analysieren. Wie in Kapitel 2 diskutiert zeigen sich auch für Milchkühe phänotypische Variationen in der Pansenmikrobiom-Zusammensetzung. Die Erforschung neuer Modellierungsansätze in genomweiten Assoziationsstudien für bakterielle oder parasitäre Mischinfektionen mit verschiedenen Erregerspezies innerhalb eines Wirtes eröffnet damit neue Möglichkeiten zur Identifizierung von Wirt-Pathogen-Interaktionen auf genomischer Ebene.

6.4 Beziehungen und Korrelationen zwischen Endoparasitenmerkmalen

Für die beiden Merkmale rFEC-GIN und rFLC-DV zeigte sich eine hohe Anzahl assoziierter Marker auf BTA 2 und 24. Da es sich bei den Nematoden GIN und *D. viviparus* um parasitäre Erreger aus der gleichen Ordnung (Strongylida) handelt, sind synergistische Effekte auf genomischer Ebene zu erwarten, wie dies bereits in den quantitativ-genetischen Studien gezeigt wurde (May et al., 2017). Als besonders interessantes Ergebnis der vorliegenden Studie ist herauszustellen, dass ein gemeinsames Kandidatengen, das Neuron navigator 3 (*NAV3*) Gen, auf BTA 5 sowohl für rFEC-GIN als auch für rFLC-DV gefunden wurde. Allerdings lag keine Übereinstimmung für die identifizierten SNPs beider Merkmale im *NAV3* Gen vor. Unterschiede in den assoziierten Markern für verschiedene Nematodenspezies, welche der gleichen biologischen Ordnung angehören, wurden auch in Scottish Blackface Schafen beobachtet (Riggio et al., 2013). Twomey et al. (2019) identifizierten ebenfalls keine übereinstimmenden genetischen Assoziationen für gleiche Merkmale verschiedener Parasitenspezies (Antikörpertiter für *Ostertagia ostertagi*, *Fasciola hepatica* und den protozoären Erreger *Neospora caninum*) oder unterschiedliche Merkmale der gleichen Parasitenspezies in irischen Milch- und Fleischrindern. Die Autoren erklären die nicht vorhandene Übereinstimmung genetischer Marker nicht nur durch spezies-spezifische Unterschiede der Erreger, sondern auch durch die niedrige genetische Korrelation zwischen den einzelnen Merkmalen auf quantitativ-genetischer Ebene. Die in der vorliegenden These verwendeten Merkmale Ei-/bzw. Larvenausscheidung von GIN und *D. viviparus* zeigten eine sehr hohe genetische Korrelation von 0,99 in den quantitativ-genetischen Analysen (May et al., 2017), überlappende Assoziationen wurden in der GWAS hingegen nicht gefunden.

Ein Ziel der Arbeit war es, basierend auf den identifizierten Markern in der GWAS gemomisch interessante Regionen (engl. Regions of interest, ROI; definiert als Kandidatengen ± 5kb) einzugrenzen und innerhalb dieser ROI SNP-Effekt Korrelationen zwischen

Endoparasitenmerkmalen und zwischen Endoparasitenmerkmalen und Produktionsmerkmalen zu schätzen. Die SNP-Effekt Korrelationen lagen zum größten Teil im moderat negativen Bereich zwischen den Merkmalen rFEC-GIN und rFEC-FH innerhalb der identifizierten ROI. Dieses Ergebnis deutet darauf hin, dass die Zucht auf eine verbesserte *F. hepatica*-Resistenz eine erhöhte Anfälligkeit für GIN-Infektionen bedingt. Zudem stimmen die SNP-Effekt Korrelationen mit Pedigree-basierten Korrelationen in vorangegangenen Studien überein, in denen genetische Korrelationen zwischen -0.10 bis 0.17 für verschiedene Merkmale einer GIN- und *F. hepatica*-Infektion geschätzt wurden (May et al., 2017; Twomey et al., 2018). Demgegenüber zeigten sich auf der überwiegenden Anzahl aller ROI für rFEC-GIN und rFLC-DV moderate bis hohe positive Korrelationen zwischen den aus der GWAS resultierenden SNP-Effekten beider Erreger. Dieses Ergebnis reflektiert sehr eindeutig den synergistischen genomischen Effekt zweier Erreger der gleichen biologischen Ordnung, welcher ursächlich in der unterschiedlichen Immunantwort zwischen einzelnen Parasiten begründet liegt. Die bovine Immunantwort im Zuge von *F. hepatica*-Infektionen ist charakterisiert durch eine zellulär vermittelte Immunantwort (Zytokine wie Interleukin (IL)-4, IL-10), wohingegen sich GIN und *D. viviparus*-Infektionen durch eine humorale Immunantwort (z.B. IgA, IgE, IgG, IgM) im Wirt auszeichnen (Foster und Elsheikka, 2012).

6.5 Beziehungen und Korrelationen zwischen Endoparasitenmerkmalen mit der Milchleistung und der Eutergesundheit

Zwischen den Merkmalen rFEC-GIN bzw. rFEC-FH und rMY wurden für die Mehrzahl der ROI hohe negative Korrelationen zwischen den SNP-Effekten geschätzt. Dieses Ergebnis ist in Übereinstimmung zu den quantitativ-genetischen Vorstudien auf Basis von Pedigree-Daten, welche primär negative genetische Korrelationen zwischen Endoparasiten- und Leistungsmerkmalen ergaben (May et al., 2017). Auch für die Merkmale rFLC-DV und rMY waren die SNP-Effekt Korrelationen negativ für die Mehrzahl aller ROI und sprechen dafür, dass eine Selektion auf hohe Milchleistung nicht mit einer erhöhten Anfälligkeit für *D. viviparus*-Infektionen einherzugehen scheint. SNP-Effekt Korrelationen zwischen den Merkmalen rFEC-GIN und rSCS waren für die meisten der identifizierten ROI negativ, wohingegen in den quantitativ-genetischen Vorstudien eine positive Korrelation geschätzt wurde (May et al., 2017). Die negative SNP-Effekt Korrelation zwischen den Merkmalen rFEC-FH und rSCS für die genomische Region auf BTA 1, welche das *ALCAM* Gen enthält,

spiegelt die Ergebnisse der Pedigree-basierten Vorstudien wider, bei welchen eine durchgehend negative genetische Korrelation zwischen beiden Merkmalen über den Laktationsverlauf hinweg geschätzt wurde. Bei einer Selektion auf verbesserte *F. hepatica*-Resistenz (niedrigere Eizahl pro Gramm Kot) ist daher aus genetischer Sicht ein Anstieg der somatischen Zellzahl zu erwarten. Die Beziehung zwischen *F. hepatica* und der somatischen Zellzahl in der Milch ist von besonderem Interesse, da eine erhöhte Anfälligkeit für bakterielle Erreger (z.B. *Mycobacterium tuberculosis, Salmonella* spp.) bei *F. hepatica*-infizierten Kühen aufgrund der durch den Parasiten induzierten Immunsuppression im Wirt bereits nachgewiesen wurde (Lucena et al., 2017; Molina-Hernández et al., 2015). Für die Zellzahl wird, wie in Kapitel 2 dargelegt, eine enge Beziehung mit durch bakterielle Erreger induzierte Mastitiden angenommen. Gutiérrez-Gil et al. (2018) identifizierten überlappende QTL für Merkmale der Mastitisresistenz und Parasitenresistenz in einer Population von Churra-Schafen. Überlappende SNPs zwischen den drei Endoparasitenmerkmalen und SCS konnten in der GWAS in Kapitel 3 nicht identifiziert werden. SNP-Effekt Korrelationen und Allelsubstitutionseffekte zwischen Endoparasiten- und Leistungsmerkmalen in Kapitel 3 und 4 zeigen sehr eindrucksvoll, dass neben gewünschten Korrelationen auf genomischer Ebene mitunter auch negative Effekte auf die Leistung der Tiere zu erwarten sind, wenn man Merkmale der Endoparasitenresistenz innerhalb bestimmter genomischer Regionen oder Marker betrachtet. Dies sollte in zukünftigen Selektionsstrategien berücksichtigt werden, um synergistische Effekte zwischen Krankheitsresistenz und ökonomisch relevanten Leistungsmerkmalen zu erzielen.

6.6 Kandidatengene und Pathways für Endoparasitenmerkmale

Kim et al. (2014, 2015) untersuchten genomweite Assoziationen für GIN-Infektionen in einer Angus-Population, welche zuvor im Rahmen eines experimentellen Zuchtprogrammes für das *DRB1* Allel des Bovinen Major Histokompatibilitätskomplexes (MHC) vorselektiert wurden. Der MHC-Komplex umfasst eine Gruppe von Genen, welche für Proteine codieren, die bei der Immunabwehr eine wichtige Rolle spielen. Gene des MHC-Komplexes wurden bereits beim Schaf (Charon, 2004; Davies et al., 2006), beim Rind (Martinez et al., 2006) sowie bei anderen Tierarten (Kurtz et al., 2004; Schwensow et al., 2007) mit Parasitenresistenzen assoziiert. Gene des MHC-Komplexes konnten in der GWAS in Kapitel 3 hingegen nicht mit einer Endoparasitenresistenz assoziiert werden.

In der vorliegenden Arbeit wurden insgesamt vier Pathways mit einer Endoparasitenresistenz assoziiert. Das Merkmal rFEC-GIN steht mit dem ‚Estrogen signaling pathway' in Verbindung.

Studien in anderen Nutztierarten haben gezeigt, dass einige Helminthenarten wie *Taenia crassiceps* oder *Ascaris suum* in der Lange sind, die Hormonproduktion wie z.B. die Produktion von 17-ß-Östradiol im Wirt zu beeinflussen und damit das eigene parasitäre Wachstum und die Reproduktion anzuregen (Escobedo et al., 2004; Fleming, 1985). Die mit dem Merkmal rFEC-GIN assoziierten Pathways ‚Cytokine-cytokine interaction pathway' und ‚PI3K-Akt signaling pathway' sind in Funktionen der zellulären Immunantwort sowie Interaktion und Mobilisierung von Immunzellen involviert und wurden bereits mit GIN-Infektionen sowie mit dem protozoären Erreger *Neospora caninum* in Verbindung gebracht (Kim et al., 2015; Li et al., 2018). Das für *F. hepatica*-Infektionen identifizierte *ALCAM* Gen codiert für das Zelladhäsionsmolekül ALCAM/CD166 und wurde mit dem ‚Cell adhesion molecules pathway' assoziiert. Swart (2002) zeigte, dass ALCAM/CD166 eine bedeutende Rolle bei der Zelladhäsion von Immunzellen spielt (z.B. Bindung von T-Helferzellen auf aktivierte Leukozyten). Eine erhöhte Expression von Zelladhäsionsmolekülen (z.B. VCAM-1) wurde auch im Rahmen von Genexpressionstudien in entzündeten Leberzellen in *F. hepatica*-infizierten Schafen beobachtet (Ruiz-Campillo et al., 2017). Beim Menschen wurde bereits eine Vielzahl an Polymorphismen im *ALCAM* Gen identifiziert, welche mit Autoimmunkrankheiten in Verbindung stehen (Wagner et al., 2014; Zhou et al., 2011). Tao et al. (2004) entwickelten einen speziellen Microarray-Chip fürs Rind unter Berücksichtigung relevanter DNA-Marker und Gene, die mit Immun- und Hormonfunktionen assoziiert sind. Die Autoren fanden eine erhöhte Expression des *ALCAM* Gens in bovinen mononuklearen Immunzellen und berücksichtigten das Gen daher auf dem Microarray-Chip. Die in Kapitel 4 geschätzten Allelsubstitutionseffekte und Dominanzeffekte innerhalb des *ALCAM* Gens für *F. hepatica*-Resistenz und für weitere mit einer Resistenz und Robustheit assoziierter Merkmale dienten ebenfalls einer Evaluierung von SNPs im *ALCAM* Gen, welche als potentiell geeignete Marker auf einem speziell für das DSN entwickelten SNP-Chip Berücksichtigung finden können.

6.7 Allelsubstitutionseffekte für Endoparasitenmerkmale, Milchproduktionsmerkmale und weitere Resistenzmerkmale im *ALCAM* Gen

In Kapitel 4 der vorliegenden Arbeit wurden vier SNPs im *ALCAM* Gen identifiziert, welche im Rahmen zukünftiger Selektionsstrategien für eine verbesserte *F. hepatica*-Resistenz beim Rind genutzt werden könnten. Eine Substitution mit dem G Allel des SNP *ALCAMc.73+32791A>G* verringerte die Wahrscheinlichkeit für *F. hepatica*-Infektionen signifikant. Für die SNPs *ALCAMc.1017T>C* und *ALCAMc.1104+85T>C* zeichnete sich das

T Allel als dominantes und günstiges Allel hinsichtlich der Wirtsresistenz gegen Leberegel aus. Interessanterweise führte eine Allelsubstitution mit dem T Allel der SNPs *ALCAMc.1017T>C* und *ALCAMc.1104+85T>C* zu einem Anstieg der Wahrscheinlichkeit für GIN-Infektionen. Diese antagonistische Beziehung zwischen GIN und *F. hepatica* wurde auch bereits in den quantitativ-genetischen Vorstudien (May et al., 2017) sowie in den in Kapitel 3 durchgeführten SNP-Effekt Korrelationen zwischen einzelnen Endoparasitenmerkmalen beobachtet. Die Übereinstimmung der Ergebnisse aus allen drei Studien zeigt, dass der selektive Genotypisierungsansatz kombiniert mit der logistischen Regressionsanalyse eine valide Methode zur Identifizierung von SNPs mit Merkmalseffekten darstellt, insbesondere für kleine Datensätze mit dem Fokus auf niedrig prävalente Gesundheitsmerkmale. Die Methodik der logistischen Regression wurde daher auch bereits für andere Gesundheitsmerkmale angewandt (Bagheri et al., 2013; Lei et al., 2006), da sie weitestgehend unabhängig von der Merkmalsverteilung ist (Tilquin et al., 2003).

Weiterhin waren die Allelsubstitutionseffekte für die ZW der Merkmale Milchfett-% und Milchprotein-% für die drei SNPs in Exon 9 und den flankierenden intronischen Regionen des *ALCAM* Gens signifikant, sowie für den FEQ auf SNP *ALCAMc.1104+10T>A*. Für die SNPs *ALCAMc.1017T>C* und *ALCAMc.1104+85T>C* konnten die höchsten ZW für Milchfett-% und Milchprotein-% für den heterozygoten Genotyp TC gefunden werden, sowie für den Genotyp AT des SNPs *ALCAMc.1104+10T>A*. Damit scheint eine Selektion auf das für *F. hepatica*-Resistenz günstige T Allel für die SNPs *ALCAMc.1017T>C* und *ALCAMc.1104+85T>C* auf genetischer Ebene nicht mit einer Verbesserung des Milchfett- oder Milchproteingehaltes einherzugehen. Demgegenüber zeigten sich für SNP *ALCAMc.1104+10T>A* in Intron 9 günstige Allelsubstitutionseffekte sowohl für das Merkmal FH-INF als auch für die Testtagsmerkmale Milchfett-%, Milchprotein-%, FEQ und Persistenz. Dieses Ergebnis ist insbesondere für zukünftige Entwicklungen spezieller SNP Chip-Arrays für DSN mit Fokus auf die verbesserte Gesundheit bei einer gleichzeitigen Aufrechterhaltung guter Leistung von Interesse. Das G Allel für den SNP *ALCAMc.73+32791A>G* zeigte sowohl für die ZW der Testtagsmerkmale MY, SCS und MUN als auch für eine Endoparasitenresistenz (rFH-INF, rGIN-INF) einen positiven Effekt. Der Genotyp TC der SNPs *ALCAMc.1017T>C* und *ALCAMc.1104+85T>C* ist mit einer signfikanten Reduktion von *F. hepatica*-Infektionen bei einer gleichzeitigen Reduktion der Milchleistung und einem Anstieg der somatischen Zellzahl assoziiert. Somit bestätigte sich die antagonistische Beziehung aus den quantitativ-genetischen

und genomischen Analysen zwischen FH-INF als Merkmal einer *F. hepatica*-Resistenz und SCS als Indikator für Eutererkrankungen auch für die im *ALCAM* Gen identifizierten SNPs. Diese antagonistischen Beziehungen könnten wie zuvor beschrieben durch die negative genetische Korrelation zwischen verschiedenen Immunantworten einzelner pathogener Erreger im Wirt zustande kommen, insbesondere da negative genetische Korrelationen zwischen der zellulären und antikörpervermittelten Immunantwort beschrieben sind, wie in Kapitel 2 diskutiert (Heriazon et al., 2013; Thompson-Crispi et al., 2012). Die Allelsubstitutionseffekte für die ZW der Merkmale MY und PERS waren für alle vier SNPs niedrig. Ein möglicher Grund dafür kann sein, dass die identifizierten SNPs im *ALCAM* Gen nicht mit anderen Genen gekoppelt sind, welche einen signifikanten Effekt auf die Milchleistung haben. Da ein Zusammenhang zwischen einer Krankheitsanfälligkeit und der Laktationspersistenz beschrieben ist, wurde das Merkmal Laktationspersistenz mit in die Analysen einbezogen. Günstige Allelsubstitutionseffekt für die Merkmale PERS und rFH-INF zeigten sich ausschließlich für den SNP *ALCAMc.1104+10T>A*. Für den Harnstoffgehalt wurden Allelsubstitutionseffekte zwischen 0,13 und 0,25 SD geschätzt, mit den höchsten Werten in dem heterozygoten Genotyp. Wie in Kapitel 2 dargelegt ist der Harnstoffgehalt ein guter Indikator für Fruchtbarkeit und Energiedefizienz in laktierenden Kühen (Rajala-Schultz et al., 2001; Roy et al., 2011). Daher impliziert die konsequente Selektion auf Kühe mit heterozygoten Genotypen für die vier SNPs einen positiven Trend für den Harnstoffgehalt, aber negative Effekte auf Fruchtbarkeitsmerkmale.

6.8 Dominanzeffekte für Endoparasitenmerkmale, Milchproduktionsmerkmale und weitere Resistenzmerkmale im *ALCAM* Gen

In Kapitel 4 erfolgte die Schätzung von Dominanzeffekten für Endoparasitenmerkmale, Produktionsmerkmale und Resistenzmerkmale für drei Loci im *ALCAM*-Gen. Hill et al. (2008) und Wittenburg et al. (2014) beschrieben niedrigere Dominanzanteile für moderat und hoch erbliche Merkmale im Vergleich zu den in der Regel niedrig erblichen funktionalen Merkmalen, was mit den Dominanzeffekt-Schätzungen aus Kapitel 4 bestätigt werden konnte. Niedrigere Dominanzanteile an der phänotypischen Varianz wurden für die hoch erblichen Produktionsmerkmale geschätzt, d.h. ZW für MY, Milchfett-% und Milchprotein-% und FEQ (d = -0,06 to 0,05). Niedrigere Heritabilitäten für funktionale Merkmale, wie Merkmale von *F. hepatica*-Infektionen (McClure et al., 2014; Twomey et al., 2018b, 2019) und GIN-Infektionen (Coppieters et al., 2009; Twomey et al., 2018b) wurden bereits in Kapitel 1 und in

weiteren Kapiteln diskutiert. Die in Kapitel 4 berechneten hohen Dominanzeffekte für rFH-INF (*d* = -2,72 to -1,69) und rGIN-INF (*d* = 0,20 to 0,75) reflektieren daher die niedrigen additiv-genetischen Varianzen für Endoparasitenmerkmale. Jiang et al. (2017) schätzten jedoch höhere Dominanzanteile für Produktionsmerkmale (z.B. Milchleistung) im Vergleich zu niedriger erblichen Fruchtbarkeitsmerkmalen.

Marshall et al. (2013) schätzten Dominanzeffekte zwischen 0,12 und 0,18 SD für QTL, welche mit Merkmalen einer Infektion mit der GIN-Spezies *Haemonchus contortus* in African Red Masaai and Dorper Schafen assoziiert waren. Die geschätzten Dominanzeffekte von Marshall et al. (2013) reflektieren die in dieser These geschätzten Werte von 0,08 bis 0,30 annäherungweise. Pant et al. (2011) untersuchten Dominanzeffekte basierend auf SNP-Daten im bovinen peptidoglycan recognition protein 1 (*PGLYRP1*) Gen, welches mit einer Resistenz gegen Paratuberkulose, induziert durch *Mycobacterium avium* subspecies, assoziiert wird. Die Autoren untersuchten in ihrer Studie ebenfalls drei SNPs im *PGLYRP1* Gen, fanden jedoch keine Assoziation zwischen den geschätzten Dominanzeffekten und Merkmalen einer Paratuberkulose-Infektion. Die Dominanzeffekte für die ZW des Merkmals SCS waren niedrig (*d* = 0,003 to 0,01) für alle drei SNPs *ALCAMc.1017T>C*, *ALCAMc.1104+10T>A* und *ALCAMc.1104+85T>C* im *ALCAM* Gen. He et al. (2011) identifizierten ebenfalls niedrige Dominanzeffekte zwischen *d* = -0,012 bis 0,0008 für das Merkmal SCS in den Immungenen *CD4* und *STAT5b*. Die in unserer Studie geschätzten Dominanzeffekte für die ZW der Merkmale Milchfett-% und Milchprotein-% waren mit Werten zwischen *d* = 0,02 bis 0,05 vergleichbar zu vorangegangenen Studien beim Holstein Rind mit Fokus auf das *DGAT1* Gen (Bovenhuis et al., 2015; Pasandideh et al., 2015). In klassischen Zuchtprogrammen wird Dominanzvariationen keine Berücksichtigung geschenkt, unter anderem aufgrund der geringen Schätzgenauigkeit für Dominanzeffekte (Ertl, 2018). Mit der Verfügbarkeit hochdichter Markerdaten eröffnen sich jedoch neue Möglichkeiten für die Rinderzucht, gezielt Dominanzeffekte für einzelne Genorte zu schätzen und in die genomische Selektion einzubeziehen. Jiang et al. (2017) untersuchten Imprinting-Effekte und Dominanzeffekte für Produktionsmerkmale, Fruchtbarkeitsmerkmale und die produktive Lebensdauer. Die Autoren schätzten signifkante Dominanzeffekte für die Produktionsmerkmale Milchleistung, Fett-kg und Protein-kg und wiesen darauf hin, nicht-additiv genetische Effekte in der genomischen Selektion zu berücksichtigen, um eine Verbesserung in den Schätzgenauigkeiten zu erzielen.

6.9 Züchterische Möglichkeiten basierend auf imputeten Daten und Vollgenomsequenzdaten

Die in Kapitel 3 durchgeführte GWAS erfolgte auf Basis imputeter Genotypen. Dabei wurden die Tiere zunächst mit dem Illumina 50K Bead Chip genotypisiert und in einem anschließenden Schritt auf HD Bead Chip Level (700K) imputet. Unter Verwendung der imputeten Genotypen konnten für alle drei Endoparasitenmerkmale weitere assoziierte Varianten identifiziert werden, welche unter ausschließlicher Verwendung der 50K Genotypen nicht abgedeckt wurden. Twomey et al. (2019) führten genomweite Assoziationsstudien für Merkmale endoparasitärer Infektionen durch und verglichen die identifizierten Marker bei Verwendung von imputeten Genotypen mit den identifizierten Markern unter Verwendung eines 50K oder HD Chips. Bei ausschließlichem Einbezug der auf dem HD Chip liegenden SNPs identifizierten Twomey et al. (2019) für das Merkmal „Leberschädigung durch *F. hepatica*“ lediglich 4 % aller QTL, welche mit den imputeten Genotypdaten nachgewiesen werden konnten. Auch bei der Verwendung des 50K Chips konnten ähnliche Varianten wie mit den imputeten Genotypdaten gefunden werden, jedoch blieb die Mehrzahl der mittels imputeter Genotypdaten identifizierten Marker bei ausschließlicher Verwendung der SNPs vom 50K Chip unentdeckt. Eine ähnliche Beobachtung zeigte sich auch für einen Abgleich assoziierter SNPs bei Verwendung der 50K Genotypen oder imputeter Genotypen in der vorliegenden Studie (Ergebnisse der GWAS mit 50K Genotypen nicht dargestellt). Rupp et al. (2015) und Gutiérrez-Gil et al. (2018) konnten für Merkmale einer Mastitisresistenz beim Schaf zeigen, dass durch die Kombination von Genotypdaten aus SNP-Chip Arrays in Verbindung mit einer Gesamtgenomsequenzierung eine höhere Genauigkeit erzielt werden kann, um kausale Mutationen zu identifizieren. Höglund et al. (2014) und Sahana et al. (2014) zeigten die bessere Genauigkeit zur Identifizierung von QTL bei Verwendung von Vollgenomsequenzdaten im Vergleich zu SNP-Chip Array-Daten (50K oder HD) für Fruchtbarkeitsmerkmale und SCS bei Milchkühen. Auch für die Feinkartierung von Erbfehlern und komplexen Gesundheitsmerkmalen kommen Vollgenomsequenzdaten inzwischen zur Anwendung (Daetwyler et al., 2014). Die Nutzung von Vollgenomsequenzdaten bietet daher auch Protential, um Merkmale der Endoparasitenresistenz feiner und über das gesamte Genom abbilden zu können. Weiterhin können die darüber identifizierten Marker in der zukünftigen Entwicklung spezieller, auf eine verbesserte Krankheitsresistenz ausgerichteter SNP-Chips berücksichtigt werden.

Vollgenomsequenzdaten können weiterhin in der vergleichenden Genomik, d.h. dem Vergleich der genomischen Charakteristika (Gene, DNA-Sequenz) verschiedener Spezies, genutzt werden. Vergleichende genomische Analysen zwischen Rindern und weiteren Tierarten sind in den vergangenen Jahren für verschiedenste genomische Regionen sowie auf Basis von Vollgenomsequenzdaten durchgeführt worden (Williams et al., 2003; Everts-van der Wind et al., 2005). Kim et al. (2012) nutzten einen innovativen neuen Ansatz aus vergleichenden genomischen Analysen in Kombination mit Heritabilitätsschätzungen für definierte Chromosomensegmente unter Einbezug humaner und prociner Sequenzdaten, um genomische Varianten für Merkmale einer Adipositas mit höherer Genauigkeit identifizieren zu können. Übertragen auf Endoparasitenmerkmale könnte ein solcher Ansatz dafür genutzt werden, um die Genauigkeit zur Identifizierung von Kandidatengenen für niedrig prävalente Parasitenspezies zu verbessern. *Strongyloides papillosus* spielt als Parasit bei Wiederkäuern eine Rolle, mit deutlich höheren Prävalenzen beim Schaf im Vergleich zum Rind (Raue et al., 2016). Eine vergleichende genomische Analyse unter Einbezug genomischer Daten von Rind und Schaf könnte damit eine Identifizierung von Kandidatengenen für eine *Strongyloides papillosus*-Resistenz erlauben, welche unter alleiniger Verwendung von Rindersequenzdaten aufgrund der geringen Prävalenz des parasitären Erregers nur sehr schwierig zu analysieren wären. Umgekehrt sind vergleichende genomische Analysen auch interessant für solche Parasitenspezies, welche sich in einigen Wirten besser etablieren können als in anderen Wirten. Für Infektionen mit dem großen Leberegel (*Fasciola hepatica*) wurde in zahlreichen Studien gezeigt, dass Schafe im Vergleich zu Rindern empfänglicher sind. Rinder besitzen Abwehrmechanismen, wie die Kalzifizierung der Gallengänge oder Bildung bestimmter Antikörper, welche in Schafen nur unzureichend vorhanden sind (Hillyer et al., 1996). Vergleichende genomische Analysen könnten damit genetische Mechanismen aufdecken, welche ursächlich zu phänotypischen Variationen in der *Fasciola hepatica*-Wirt-Interaktion zwischen Schafen und Rindern beitragen.

6.10 *Fasciola hepatica* Seroprävalenzen und phänotypische Assoziationsanalysen mit Milchproduktionsmerkmalen

In Kapitel 5 wurden *F. hepatica*-Seroprävelenzen der Jahre 2017 und 2018 in norddeutschen Milchviehherden untersucht und mit zuletzt publizierten Seroprävelenzen der Jahre 2010, 2008 und 2006 aus den Studien von Kuerpick et al. (2012a, 2012b, 2013) verglichen. Zudem sollten Assoziationsanalysen zwischen *F. hepatica*-Antikörpertitern und Milchleistungsparametern

sowie der somatischen Zellzahl Aufschluss darüber geben, wie die wirtschaftliche Relevanz des Erregers in deutschen Milchviehherden einzustufen ist. Die in Kapitel 5 durchgeführten phänotypischen Assoziationsanalysen zwischen *F. hepatica*-Herdenantikörpertitern und Milchproduktionsparametern der Herde ergaben einen Verlust von -1,62 kg Milch pro Kuh pro Tag zwischen Herden in der höchsten Antikörpertiter-Kategorie sowie negativen Herden (Antikörpertiter ≤ 30 S/P %). Dieses Ergebnis ist in Übereinstimmung zu bisherigen Studien, welche Verluste bis zu 4,4 kg pro Kuh pro Tag schätzten (Charlier et al., 2007; Mezo et al., 2011). Wie in den Kapiteln 3 und 4 diskutiert, wird eine erhöhte Anfälligkeit für bakterielle Mastitiserreger durch *F. hepatica*-Infektionen vermutet, da *F. hepatica* die Immunantwort im Wirt unterdrückt (Molina-Hernández et al., 2015). Die auf genomischer und molekulargenetischer Ebene beobachtete antagonistische Beziehung zwischen dem Merkmal *F. hepatica*-Eiausschiedung und SCS konnte in den phänotypischen Assoziationsanalysen auf Herdenebene nicht bestätigt werden, zudem zeigte sich die Tendenz von niedrigeren durchschnittlichen Zellzahlen in niedrig infizierten Herden im Vergleich zu stark infizierten Herden. Die *F. hepatica*-Antikörpertiter-Kategorie hatte keinen signifikanten Effekt auf die durchschnittliche somatische Zellzahl der im Modell einbezogenen Milchviehherden. Jedoch ist zu erwähnen, dass in Kapitel 3 und 4 das Merkmal „*F. hepatica*-Eiausscheidung“ als Indikatormerkmal für eine Resistenz auf Basis von Einzeltiermessungen verwendet wurde, wohingegen in Kapitel 5 das Merkmal „*F. hepatica*-Anitkörpertiter“ auf Herdenebene betrachtet wurde. In der vorliegenden Arbeit betrugen die geschätzten Einbußen im Milchprotein- und Milchfettgehalt 0,06 kg pro Kuh pro Tag zwischen der höchsten Antikörpertiter-Kategorie und ELISA-negativen Herden. Änderungen im Milchprotein- und fettgehalt können sich durch eine negative Energiebilanz ergeben (Reist et al., 2002), welche aus einer Reduktion der Futteraufnahme aufgrund von *F. hepatica*-Infektionen resultieren kann. Dieses Ergebnis reflektiert die hohe wirtschaftliche Relevanz von *F. hepatica*-Infektionen in Milchviehherden und die Notwendigkeit zur Identifizierung genomischer Marker für eine *F. hepatica*-Resistenz, welche im Rahmen zukünftiger genomischer Selektionsstrategien genutzt werden können. Dies ist insbesondere von Bedeutung, da die Behandlungsmöglichkeiten gegen *F. hepatica* durch die begrenzte Verfügbarkeit wirksamer Anthelmintika sehr stark eingeschränkt sind. Der Wirkstoff Triclabendazol, welcher gegen juvenile und adulte Leberegel effektiv wirkt, ist ausschließlich für nicht laktierende Tiere zugelassen (vor dem 41. Tag vor der Kalbung). Oxyclozanid wirkt hingegen mit einer Wartezeit

von 4,5 Tagen für laktierende Tiere ausschließlich gegen adulte Egel. Dass die derzeitigen Behandlungsmöglichkeiten der Fasciolose sehr begrenzt sind zeigt sich deutlich in den Seroprävalenzen mit 33,1 % positiver Tankmilchproben in 2017 und 37,0 % positiver Tankmilchproben in 2018 in der niedersächsischen Region Ostfriesland. Die Tankmilchproben der vorliegenden Arbeit wurden zwischen den Monaten Oktober und Dezember untersucht, wobei über 70 % aller Proben in den Monat November fielen. Damit liegen die Seroprävalenzen niedriger als in den Studien von Kuerpick et al. (2012a, 2012b) zwischen den Jahren 2006 und 2010, welche Seroprävalenzen zwischen 48 und 54 % detektierten. Gründe für die niedrigeren Serorpävalenzen können zum einen durch das Studiendesign bedingt sein. Zum anderen begründen sich die niedrigeren Seroprävalenzen womöglich auch darin, dass die Landwirte bereits in den früheren Studien über das Problem der Fasciolose in ihren Betrieben informiert waren und entsprechende Kontrollmaßnahmen zum Weidemanagement ergriffen haben, was zu einer Reduktion der Infektionsraten und damit zu einem Absinken der Serorpävalenzen in 2017 und 2018 führte. Klimatische Bedingungen spielen eine große Rolle für die Entwicklung von *F. hepatica* im Zwischenwirt, der Zwergschlammschnecke *Galba truncatula*. Das Jahr 2018 ist laut Angaben des Deutschen Wetterdienstes das im Durchschnitt wärmste (Durchschnittstemperatur: 10,5° C) und vierttrockenste Jahr seit 1881 gewesen, und auch in 2019 war eine erhebliche Trockenheit zu verzeichnen. Daher lässt sich vermuten, dass die Seroprävalenzen in Deutschland in den folgenden Jahren aufgrund der schlechten Bedingungen für den Zwischenwirt und die Entwicklung von *F. hepatica* tendenziell sinken werden.

6.11 Phänotypische Assoziationsanalysen zwischen *F. hepatica*-Infektionen und Milchketonkörpern

Kapitel 5 der vorliegenden Arbeit betrachtet erstmals die Beziehung zwischen dem *F. hepatica*-Antikörpertiterstatus in Milchviehherden und den Milchketonkörpern BHB und Aceton, abgeleitet mittels FTIR Spektrometrie aus der routinemäßigen Milchuntersuchung. Wie in Kapitel 2 diskutiert werden die aus der FTIR Spektrometrie abgeleiteten Milchketonkörper derzeit bereits in vielen Ländern zur Herdendiagnostik primärer Ketosen in Milchviehbetrieben genutzt (Schwarz et al., 2015) und finden auch in der Zucht als neue innovative Merkmale Verwendung (Koeck et al., 2014). Belay et al. (2017) und Koeck et al. (2014) schätzten jedoch nur niedrige phänotypische Korrelationen zwischen im Blut und in der Milch gemessenen BHB-Werten mit durch den Landwirt erfassten Ketosediagnosen. Die Übereinstimmung zwischen eingetragenen Ketosediagnosen der Landwirte und den mittels FTIR Spektrometrie

abgeleiteten BHB-Werten ist ebenfalls gering mit einem hohen Anteil an Tieren mit erhöhten BHB-Werten, aber keinerlei Auffälligkeiten für eine primäre Ketose (unpublizierte Ergebnisse aus eigenen Analysen). Eine Erklärung dafür kann, wie in Kapitel 2 angedeutet, eine schlechte Dokumentation von Ketosefällen durch die Landwirte in der Praxis sein (Zwald et al., 2004). Als einen weiteren Grund für erhöhte Milchketonkörper bei gleichzeitiger Abwesenheit klinischer Anzeichen einer primären Ketose wurde in der vorliegenden Arbeit die Hypothese aufgestellt, dass *F. hepatica*-Infektionen durch eine reduzierte Futteraufnahme sekundär zu einer Reduktion des Leberglucosegehaltes führen und damit eine Erhöhung von Ketonkörpern bedingen. Der Einfluss von *F. hepatica*-Infektionen auf Glucose- und BHB-Gehalt im Serum wurde beim Schaf bereits in zahlreichen Studien nachgewiesen (z.B. Kozat und Denizhan, 2010; Phiri et al., 2007). Die einzige bis dato beim Rind durchgeführte Studie zeigte keinen signifikanten Unterschied in Serum-Glucose sowie BHB-Werten zwischen patent *F. hepatica*-infizierten und nicht-infizierten Kühen (Kowalczyk et al., 2018). Da Rinder im Vergleich zu Schafen resistenter gegen *F. hepatica* sind (durch eine Kalzifizierung der Gallengänge) (Hillyer et al., 1996), fallen Sekundäreffekte durch patente *F. hepatica*-Infektionen wie z.B. eine Erhöhung der Leberenzyme oder Ketonkörper vermutlich geringer aus. Der *F. hepatica*-Herden-Antikörpertiterstatus zeigte einen signfikanten Effekt auf die durchschnittlichen Milch-BHB-Werte in der vorliegenden Arbeit. Stark infizierte Herden hatten signifkant höhere Werte des Milchketonkörpers BHB im Vergleich zu Herden in den drei niedrigeren Infektionsklassen. Dieser signifkante Effekt konnte für den Ketonkörper Aceton nicht gezeigt werden, auch wenn die Acetongehalte in stark infizierten Herden im Vergleich zu nicht infizierten Herden durchschnittlich höher lagen. Die Ergebnisse der vorliegenden Analysen veranschaulichen sehr deutlich den Zusammenhang zwischen infektiösen Erkrankungen und Indikatormerkmalen wie z.B. Milchketonkörpern, welche bisher ausschließlich unter dem Aspekt primärer Stoffwechselerkrankungen analysiert wurden. Auf Basis der Ergebnisse dieser These lässt sich ableiten, dass erhöhte Milchketonkörper bei vielen Einzeltieren als auch in vielen Milchviehherden neben primären Ketosen auch auf weitere Erkrankungen wie die Fasciolose zurückgeführt werden können, welche sekundäre Stoffwechselerkrankungen bedingen. Dennoch sollten die Ergebnisse dieser Arbeit auf Herdenebene in weiteren Studien auf Einzeltierebene bestätigt werden. Der Zusammenhang zwischen Fasciolose und Milchketonkörpern ist insbesondere deshalb von Interesse, da Milchketonkörper wie in Kapitel 2 beschrieben bereits in einigen Ländern als Indikatormerkmale für Stoffwechselerkrankungen

in den Selektionsindex einfließen. Eine fälschlicherweise falsch positive Deklaration von Tieren als „primäre Ketosetiere“, welche jedoch tatsächlich unter sekundären Ketosen aufgrund von Fasiolose leiden, ist daher nicht auszuschließen. Die in Kapitel 2 vorgestellten Indikatormerkmale bzw. Bioindikatoren für Erkrankungen bei der Milchkuh sind daher immer im Gesamtkontext mit anderen Differentialdiagnosen zu betrachten.

6.12 Praktische Aspekte der Zucht auf Endoparasitenresistenz und Forschungen zur Wirt-Parasiten-Interaktion

Ziel der vorliegenden Arbeit war es, den quantitativ-genetischen, genomischen und molekulargenetischen Hintergrund für endoparasitäre Infektionen beim Milchrind zu beleuchten. Infektionen mit GIN, *D. viviparus* und *F. hepatica* spielen insbesondere in der Weidehaltung eine Rolle, da es sich bei allen drei Erregern in erster Linie um Weideinfektionen handelt. Weidebasierte Produktionssysteme werden sowohl von konventioneller als auch von ökologischer Seite in den vergangenen Jahren wieder vermehrt zur Milcherzeugung genutzt. Insbesondere im Zuge der öffentlichen Diskussionen zu Tierschutz und Tierwohl erfreut sich die Weidehaltung auch bei Verbrauchern einer immer größeren Beliebtheit. Im Gegensatz zur reinen Stallhaltung verlangt die Weidehaltung Milchkühen jedoch andere Eigenschaften wie eine gute Robustheit, eine verbesserte Toleranz und Widerstandsfähigkeit gegenüber Schwankungen in den Haltungsbedingungen ab. Funktionale Merkmale wie die verbesserte Endoparasitenresistenz rücken daher wieder verstärkt in den Fokus tierzüchterischen Interesses. Rassen, denen eine besondere Robustheit an die rauen Umweltbedingungen auf der Weide nachgesagt wird, gewinnen in diesem Zuge wieder verstärkt an Bedeutung. Kapitel 3 und 4 der vorliegenden Arbeit fokussierten daher auf das DSN als eine vom Aussterben bedrohte Rasse mit kleiner Populationsgröße, welche in vielen Teilen Deutschlands aufgrund der guten Anpassung an Weideproduktionsbedingungen genutzt wird (Al-Kanaan, 2016; Jaeger et al., 2016). Da kleine Populationen wie das DSN den größeren Milchrindpopulationen in vielen Leistungsmerkmalen nachstehen, ist die Analyse charakteristischer Merkmale wie z.B. Endoparasitenresistenz, welche in Weidehaltung eine besondere Rolle spielen, für den Erhalt solcher Rassen von großer Relevanz. Für das Angus-Rind sind daher bereits Anfang der 90er Jahre intensive Studien betrieben worden, um auf Nachkommen mit einer verbesserten Endoparasitenresistenz zu selektieren. Gasbarre et al. (1995) konnten Unterschiede zwischen den Nachkommen für das Merkmal Eizahl pro Gramm Kot (EpG) gastrointestinaler Nematoden zwischen verschiedenen Angus-Bullen nachweisen. In weiteren Selektionsexperimenten war es

basierend auf Information der Väter möglich, Nachkommen zu züchten, welche durchgehend niedrige EpG-Werte oder deutlich reduzierte EpG-Werte nach einer Reinfektion mit GIN aufwiesen (Gasbarre et al., 2001). Die Selektion auf Tiere mit verminderter Eiausscheidung kann insbesondere in Weidehaltung den Infektionsdruck auf der Weide stark reduzieren (McCanus et al., 2014). Eine umfangreiche Phänotypisierung von Tieren für endoparasitäre Infektionen ist in der Praxis aufgrund des Aufwandes und der Kosten jedoch nicht praktikabel und erschwert damit die Selektion.

Mit der Verfügbarkeit von SNP-Daten und engmaschigen genomischen Daten eröffnen sich neue züchterische Möglichkeiten, Krankheitsresistenz und Robustheit beim Rind zu verbessern. In Kapitel 3 und 4 der vorliegenden Arbeit konnten SNP-Marker beim DSN identifiziert werden, welche mit den Merkmalen Ei-bzw. Larvenausscheidung von Endoparasiten assoziiert sind und in immunologische Abwehrmechanismen des Wirtes involviert sind, was mittels GWAS und anschließender Pathway-Analysen nachgewiesen werden konnte. Allerdings hat sich in der vorliegenden Arbeit wie auch in Studien von Twomey et al. (2018, 2019) gezeigt, dass die identifizierten Marker und Pathways sehr spezifisch je nach Parasitenspezies sind. Guasconi et al. (2012) und Zintl et al. (2015) konnten belegen, dass Infektionsmerkmale nicht nur von der genetisch bedingten Wirtsresistenz, sondern auch von der Genetik des parasitären Erregers bestimmt werden. Analysen, welche genetische und immunologische Mechanismen der Wirt-Parasiten-Interaktion unter Berücksichtigung des Genoms beider Interaktionspartner erforschen, liegen für Infektionen mit den Helminthen GIN, *F. hepatica* und *D. viviparus* noch nicht vor. Methoden des Next-Generation-Sequencing (NGS) ermöglichen es, auch Erregergenome vollständig zu sequenzieren und miteinander zu vergleichen (Wit und Gilleard, 2017). Mit einer Verknüpfung genomischer Daten beider Interaktionspartner ist es daher möglich, Resistenzmechanismen im Wirt in Abhängigkeit des Parasitengenoms zu modellieren und Unterschiede in der Resistenz verschiedener Wirte für verschiedene Parasiten-Genotypen abzuleiten. Dieses Wissen kann in einem weiteren Schritt für die gezielte Entwicklung von Impfstoffen und Anthelmintika genutzt werden. Neue Methoden des NGS wie „Dual RNA-seq“ ermöglichen eine gezielte und kombinierte Analyse der Genexpression auf Basis von RNA-Sequenzierungen für Wirt und Pathogen (Westermann et al., 2012). Sequenzierungsmethoden der dritten Generation wie der MinION der Firma Oxford Nanopore Technologies ermöglichen eine zielgerichtete Analyse bakterieller, viraler und parasitärer Genome im Hochdurchdatzverfahren nicht nur im Labor, sondern auch im Feld (D’Avila-Levy

et al., 2019; Kilianski et al., 2015). Auch bei Nutztieren wird diese Technologie bereits erfolgreich angewendet, um Genome verschiedener Erregerspezies zu sequenzieren (Hansen et al., 2019; McCabe et al., 2018). Neue Technologien des NGS und Modellierungen unter gleichzeitigem Einbezug des Wirts- und Parasitengenoms revolutionieren damit die Forschung und bieten neue Möglichkeiten, Mechanismen von Wirt-Pathogen-Interaktionen abzuleiten und zu verstehen.

Schlussfolgerungen der Arbeit

Endoparasitäre Infektionen sind ein bestehendes Problem mit wirtschaftlicher Relevanz in Milchviehherden, insbesondere in Weideproduktionssystemen. SNP-Marker können in der Zucht mit dem Ziel auf eine verbesserte Krankeitsresistenz und Robustheit genutzt werden. Insbesondere für vom Aussterben bedrohte Rassen mit kleiner Populationsgröße kann eine Selektion auf bestimmte Resistenzmerkmale wie Endoparasitenresistenz von Vorteil sein, wie dies in der vorliegenden Arbeit beispielhaft an einer Population des DSN untersucht wurde. Weitere Schlussfolgerungen lassen sich aus der vorliegenden Arbeit ableiten:

- Bei Milchkühen sind die Heritabilitäten für das Merkmal „GIN-Eiausscheidung“ und „*D. viviparus*-Larvenausscheidung“ niedrig, wohingegen die Heritabilität des Merkmals „*F. hepatica*-Eiausscheidung“ im moderaten Bereich liegt. Negative genetische Korrelationen zwischen *F. hepatica* mit der Milchleistung und dem Milchproteingehalt zeigen, dass eine Selektion auf verbesserte *F. hepatica*-Resistenz nicht mit Leistungseinbußen bei Milchkühen assoziiert ist.
- Die Berücksichtigung von Immunmerkmalen in Selektionsstrategien für eine verbesserte Krankheitsresistenz beim Rind wirkt sich, je nach Immunantwort des endoparasitären Erregers, positiv oder negativ auf eine verbesserte Endoparasitenresistenz im Wirt aus.
- Die in eine Endoparasitenresistenz beteiligten Gene sind sehr pathogenspezifisch. Die in eine Endoparasitenresistenz involvierten immunologischen Mechanismen schließen insbesondere Vorgänge der Zelladhäsion und Interaktion zwischen Immunzellen wie T- und B-Zellen ein.

- Der zweistufige Analyseansatz für die GWAS mit einer Vorkorrektur endoparasitärer Phänotypen in einem großen Datensatz und der anschließenden Verwendung der Residuen in einem kleineren Datensatz stellt eine valide methodische Vorgehensweise zur Identifizierung assoziierter SNP-Marker dar, insbesondere für vom Aussterben bedrohte Rassen mit kleiner Populationsgröße wie das DSN.
- Das Activated leukocyte cell adhesion molecule (*ALCAM*) Gen ist mit einer *F. hepatica*-Resistenz beim DSN assoziiert. Signifikante Allelsubstitutionseffekte zeigten sich in vier SNPs in Exon 9 und den flankierenden intronischen Regionen des *ALCAM* Gens für das Merkmal „*F. hepatica*-Eiausscheidung“.
- Die in den genomischen und molekulargenetischen Analysen geschätzten antagonistischen Effekte zwischen *F. hepatica*-Resistenz und der somatischen Zellzahl in der Milch konnten in phänotypischen Analysen auf Herdenebene nicht bestätigt werden.
- In den Jahren 2017 und 2018 konnte ein um 10-20 %-iger Rückgang der *F. hepatica*-Seroprävalenzen im Vergleich zu Seroprävalenzstudien aus den Jahren 2006-2010 in ostfriesischen Milchviehherden beobachtet werden, der sich ursächlich vermutlich auf Änderungen der klimatischen Bedingungen zurückführen lässt. Dennoch stellen *F. hepatica*-Infektionen ein bestehendes Problem in norddeutschen Milchviehherden dar mit derzeit ca. 1/3 infizierter Betriebe und nachgewiesenen signifikanten Einbußen in der Milchleistung.
- Eine Erhöhung der Milch BHB-Werte in Milchviehherden kann ein Indiz für bestehende oder vergangene *F. hepatica*-Infektionen sein und sollte als Differentialdiagnose für primäre Ketosen in Betracht gezogen werden. Indikatormerkmale als Biomarker für infektiöse oder nicht-infektiöse Erkrankungen sind immer in einem physiologischen Gesamtkontext und unter Berücksichtigung weiterer Differentialdiagnosen zu betrachten.

Referenzen

Al-Kanaan A. 2016. Heat stress response for physiological traits in dairy and dual-purpose cattle populations on phenotypic and genetic scales. PhD thesis, Faculty of Organic Agriculture, University of Kassel.

Bagheri M, Miraie-Ashtiani R, Moradi-Shahrbabak M, Nejati-Javaremi A, Pakdel A, von Borstel UU, Pimentel ECG, König S. 2013. Selective genotyping and logistic regression analyses to identify favorable SNP-genotypes for clinical mastitis and production traits in Holstein dairy cattle. Livestock Science 151:140-151.

Banks BD, Mao IL, Walter JP. 1985. Robustness of the restricted maximum likelihood estimator derived under normality as applied to data with skewed distributions. Journal of Dairy Science 68:1785-1792.

Belay TK, Svendsen M, Kowalski ZM, Ådnøy T. 2017. Genetic parameters of blood ß-hydroxybutyrate predicted from milk infrared spectra and clinical ketosis, and their associations with milk production traits in Norwegian Red cows. Journal of Dairy Science 100:6298-6311.

Bishop SC, Woolliams JA. 2012. On the genetic interpretation of disease data. PLoS ONE 5:e8940.

Bohmanova J, Misztal I, Tsuruta S, Norman HD, Lawlor TJ. 2008. Short Communication: Genotype by environment interaction due to heat stress. Journal of Dairy Science 91:840-846.

Bovenhuis H, Visker MHPW, van Valenberg HJF, Buitenhuis AJ, Arendok JAM. 2015. Effects of the *DGAT1* polymorphism on test-day milk production traits throughout lactation. Journal of Dairy Science 98:6572-6582.

Brownie C, Boos DD, Oliver JH. 1990. Modifying the t and ANOVA F tests when treatment is expected to increase variability relative to controls. Biometrics 46:259-266.

Brügemann K, Gernand E, von Borstel UU, König S. 2011. Genetic analyses of protein yield in dairy cows applying random regression models with time-dependent and temperature x humidity-dependent covariates. Journal of Dairy Science 94:4129-4139.

Charlier J, Duchateau L, Clearebout E, Williams D, Vercruysse J. 2007. Associations between anti-*Fasciola hepatica* antibody levels in bulk-tank milk samples and production parameters in dairy herds. Preventive Veterinary Medicine 78:57-66.

Charon KM. 2004. Genes controlling resistance to gastrointestinal nematodes in ruminants. Animal Science Papers and Reports 22:135-139.

Coppieters W, Mes THM, Druet T, Farnir F, Tamma N, Schrooten C, Cornelissen AWCA, Georges M, Ploeger HW. 2009. Mapping QTL influencing gastrointestinal nematode burden in Dutch Holstein-Friesian dairy cattle. BMC Genomics 10:96.

Davies G, Stear MJ, Benothman M, Abuagob O, Kerr A, Mitchell A, Bishop SC. 2006. Quantitative trait loci associated with parasitic infection in Scottish blackface sheep. Heredity 96:252-258.

D'Avila-Levy CM, Bearzatto B, Ambroise J, Helaers R, Butenko A, Yurchenko V, Morelli KA, Santos HLC, Brouillard P, Grellier P, Gala JL, Vikkula M. 2019. First Draft of the trypanosomatid *Herpetomonas muscarum ingenoplastis* through MinION Oxford Nanopore Technology and Illumina Sequencing. Preprints:2019199271.

De Jong G, Bijma P. 2002. Selection and phenotypic plasticity in evolutionary biology and animal breeding. Livestock Production Science 78:195-214.

Ertl J. 2018. Investigations on genomic evaluations using high-density genotypes in Fleckvieh with consideration of dominance effects. Technische Universität München, Dissertation.

Escobedo EG. 2004. Molecular mechanism involved in the differential effects of sex steroids on the reproduction and infectivity of *Taenia crassiceps*. Journal of Parasitology 90:1235-1244.

Everts-van der Wind A, Larkin DM, Green CA, Elliott JS, Olmstead CA, Chiu R, Schein JE, Marra MA, Womack JE, Lewin HA. 2005. A high-resolution whole-genome cattle-human comparative map reveals details of mammalian chromosome evolution. PNAS 102:18526-18531.

Fleming MW. 1985. *Ascaris suum*: role of ecdysteroids in molting. Experimental Parasitology 60:207-210.

Foster N, Elsheikka HM. 2012. The immune response to parasitic helminths of veterinary importance and its potential manipulation for future vaccine control strategies. Parasitology Research 110:1587-1599.

Gasbarre LC, Leighton EA, Bryant D. 1995. Genetically controlled resistance to gastrointestinal nematode infections of cattle. Proceedings Beef Improvement Federation, p212-217.

Gasbarre LC, Leighton EA, Sonstegard T. 2001. Role of bovine immune system and genome in resistance to gastrointestinal nematodes. Veterinary Parasitology 98:51-64.

Gernand E, König S. 2014. Random regression test-day model for clinical mastitis: Genetic parameters, model comparison, and correlations with indicator traits. Journal of Dairy Science 97:3953-3963.

Guasconi L, Serradell MC, Masih DT. 2012. *Fasciola hepatica* products induce apoptosis of peritoneal macrophages. Veterinary Immunology and Immunopathology 148:359-363.

Gutiérrez-Gil B, Esteban-Blanco C, Suarez-Vega A, Arranz JJ. 2018. Detection of quantitative trait loci and putative causal variants affecting somatic cell score in dairy sheep by using a 50K SNp-Chip and whole-genome sequencing. Journal of Dairy Science 101:1-17.

Häggman J, Juga J. 2013. Genetic parameters for hoof disorders and feet and leg conformation traits in Finnish Holstein cows. Journal of Dairy Science 96:3319-3325.

Hanotte O, Ronin Y, Agaba M, Nilsson P, Gelhaus A, Horstmann R, Sugimoto Y, Kemp S, Gibson J, Korol A, Soller M, Teale A. 2002. Mapping of quantitative trait loci controlling trypanotolerance in a cross of tolerant West African N'Dama and susceptible East African Boran cattle. Proceedings of the National Academy of Sciences of the United States of America 100:7443-7448.

Hansen S, Dill V, Salaby MA, Eschbaumer M, Böhlken-Fascher S, Hoffmann B, Czerny CP, Wahed AAE. 2019. Serotyping of foot-and-mouth disease virus using oxford nanopore sequencing. Journal of Virological Methods 263:50-53.

He Y, Chu Q, Ma P, Wang Y, Zhang Q, Sun D, Zhang Y, Yu Y, Zhang Y. 2011. Association of bovine *CD4* and *STAT5b* single nucleotide polymorphisms with somatic cell scores and milk production traits in Chinese Holsteins. Journal of Dairy Research 78:242-249.

Heriazon A, Quinton M, Miglior F, Leslie KE, Sears W, Mallard BA. 2013. Phenotypic and genetic parameters of antibody and dalyed-type hypersensitivity responses of lactating Holstein cows. Veterinary Immunology and Immunopathology 154:83-92.

Hillyer GV, Soler de Galanes M, Buchón P, Bjorland J. 1996. Herd evaluation by enzyme-linked immunosorbent assay for the determination of *Fasciola hepatica* infection in sheep and cattle from Altiplano of Bolivia. Veterinary Parasitology 61:211-220.

Hill WG, Goddard ME, Visscher PM. 2008. Data and theory point to mainly additive genetic variance for complex traits. PLoS Genetics 4(2):e1000008.

Hua X, Song L, Yu G, Goedert JJ, Abnet CC, Landi MT, Shi J. 2015. MicrobiomeGWAS: a tool for identifying host genetic variants associated with microbiome composition. bioRxiv.

Jaeger M, Brügemann K, König S. 2016. Genetic relationships and trait comparison between and within lines of local dual-purpose cattle. 67th Annual meeting of the European Association for Animal Production, Belfast, Irland.

Jiang J, Shen B, O'Connell JR, VanRaden PM, Cole JB, Ma L. 2017. Dissection of additive, dominance, and imprinting effects for production and reproduction traits in Holstein cattle. BMC Genomics 18:425.

Kilianski A, Haas JL, Corriveau EJ, Liem AT, Willis KL, Kadavy DR, Rosenzweig CN, Minot SS. 2015. Bacterial and viral identification and differentiation by amplicon sequencing on the MinION nanopore sequencer. GigaScience 4:12.

Kim J, Lee T, Kim TH, Lee KT, Kim H. 2012. An integrated approach of comparative genomics and heritability analysis of pig and human on obesity trait: evidence for candidate genes on human chromosome 2. BMC Genomics 13:711.

Kim ES, Sonstegard TS, Silva MV, Gasbarre LC, van Tassell CP. 2014. Identification of quantitative trait loci affecting gastrointestinal parasite resistance in an experimental Angus population. Animal Genetics 45:117-121.

Kim ES, Sonstegard TS, Silva MV, Gasbarre LC, van Tassell CP. 2015. Genome-wide scan of gastrointestinal nematode resistance in closed Angus population selected for minimized influence of MHC. PLoS One 10:e0119380.

Koeck A, Jamrozik J, Schenkel FS, Moore RK, Lefebvre DM, Kelton DF, Miglior F. 2014. Genetic analysis of milk ß-hydroxybutyrate and its association with fat-to-protein ratio, body condition score, clinical ketosis, and displaced abomasums in early first lactation of Canadian Holsteins. Journal of Dairy Science 97:7286-7292.

König S, Wu XL, Gianola D, Heringstad B, Simianer H. 2008. Exploration of relationships between claw disorders and milk yield in Holstein cows via recursive linear and threshold models. Journal of Dairy Science 91:395-406.

Kolmodin R, Strandberg E, Madsen P, Jensen J, Jorjani H. 2002. Genotype by environment interaction in Nordic dairy cattle studied using reaction norms. Animal Science 52:11-24.

Kowalczyk SJ, Czopowicz M, Weber CN, Müller E, Kaba J. 2018. Accuracy of a diagnostic model based on serum biochemical parameters in detecting cows at an increased risk of chronic fasciolosis. Veterinary Parasitology 254:15-20.

Kozat S, Denizhan V. 2010. Glucose, lipid, and lipoprotein levels in sheep naturally infected with *Fasciola hepatica*. Journal of Parsitology 96:657-659.

Kuerpick B, Schnieder T, Strube C. 2012a. Seasonal pattern of *Fasciola hepatica* antibodies in dairy herds in Northern Germany. Parasitology Research 111:1085-1092.

Kuerpick B, Fiedor C, von Samson-Himmelstjerna G, Schnieder T, Strube C. 2012b. Bulk milk-estimated seroprevalence of *Fasciola hepatica* in dairy herds and collecting of risk factor data in East Frisia, Northern Germany. Berliner und Münchener Tierärztliche Wochenschrift 125:345-350.

Kuerpick B, Conraths FJ, Staubach C, Fröhlich A, Schnieder T, Strube C. 2013. Seroprevalence and GIS-supported risk factor analysis of *Fasciola hepatica* infections in dairy herds in Germany. Parasitology 140:1051-1060.

Kurtz J, Kalbe M, Aeschlimann PB, Häberli MA, Wegner KM, Reusch TBH, Milinski M. 2004. Major histocompatibility complex diversity influences parasite resistance and innate immunity in sticklebacks. Proceedings of the Royal Society of London Series B-Biological Sciences 271:197-204.

Lei Z, Yuan-Yuan Y, Zhong-Hao L, Li-Juan K, Guan-Dong X, He-Shuang D, Gen-Liu W. 2006. Detection and characterization of PCR-SSCP markers of the bovine lactoferrin gene for clinical mastitis. Asian-Australasian Journal of Animal Science 19:1399-1403.

Li S, Gong P, Tai L, Li X, Wang X, Zhao C, Zhang X, Yang Z, Yang J, Li J, Zhang X. 2018. Extracellular vesicles secreted by *Neospora caninum* are recognized by Toll-like receptor 2 and modulate host cell innate immunity through the MAPK signaling pathway. Frontiers in Immunology 9:1633.

Lucena AN, Cuartero L, Mulcahy G, Zintl A. 2017. The immunomodulatory effects of co-infection with *Fasciola hepatica*: From bovine tuberculosis to Johne's disease. The Veterinary Journal 222:9-16.

Lynch M, Walsh B. 1998. Genetics and analysis of quantitative traits. Sinauer Associations Inc Publishers, Sunderland, MA, USA.

Mahmoud M, Zeng Y, Shirali M, Yin T, Brügemann K, König S, Haley C. 2018. Genome-wide pleiotropy and shared biological pathways for resistance to bovine pathogens. PloS ONE 13(4):e0194374.

May K, Brügemann K, Yin T, Scheper C, Strube C, König S. 2017. Genetic line comparisons and genetic parameters for endoparasite infections and test-day milk production traits. Journal of Dairy Science 100:7330-7344.

Marshall, K, Mugambi JM, Nagda S, Sonstegard TS, Van Tassell CP, Baker RL, Gibson JP. 2013. Quantitative trait loci for resistance to *Haemonchus contortus* artificial challange in Red Maasai and Dorper sheep of East Africa. Animal Genetics 44:285-295.

Martinez ML, Machado MA, Nascimento CS, Silva MVGB, Teodoro RL, Furlong J, Prata MCA, Campos AL, Guimarães MFM, Azevedo ALS, Pires MFA, Verneque RS. 2006. Association of BoLA-DRB3.2 alleles with tick (*Boophilus microplus*) resistance in cattle. Genetic Molecular Research 5:513-524.

McCabe MS, Cormican P, Johnston D, Early B. 2018. Simultaneous detection of DNA and RNA virus species involved in bovine respiratory disease by PCR-free rapid tagmentation-based library preparation and MinION nanopore sequencing.

McClure J, Cromie AR, Sayers R, Graham D, Byrne N, Berry DP. 2014. Genetics of susceptibility to bovine diarrhea virus, bovine herpesvirus type 1 and *Fasciola hepatica* in cattle. In: 10. Kongress Genetik für die Tierproduktion Vancouver, S. 542-545.

Mezo M, Gonzalez-Warleta M, Antonio Castro-Hermida J, Muino L, Ubeira FM. 2011. Association between anti-*F. hepatica* antibody levels in milk and production losses in dairy cows. Veterinary Parasitology 180:237-242.

Meyer K. 2005. Random regression analyses using B-splines to model growth of Australian Angus cattle. Genetic Selection Evolution 37:473-500.

Michel JF, Lancaster MB, Hong C. 1979. The effect of age, acquired resistance, pregnancy and lactation on some reactions of cattle to infection with *Ostertagia ostertagi*. Parasitology 79:157-168.

Misztal I, Strabel T, Mantysaari EA, Meuwissen THE, Jamrozik J. 2000. Strategies for estimating the parameters needed for different tst-day models. Journal of Dairy Science 83:1125-1134.

Molina-Hernández V, Mulcahy G, Pérez J, Martínez-Moreno Á, Donnelly S, O´Neill SM, Dalton JP, Cwiklinski K. 2015. *Fasciola hepatica*: We may not be there yet but we´re on the right road. Veterinary Parasitology 208:101-111.

Morris CA, Green RS, Cullen NG, Hickey SM. 2003. Genetic and phenotypic relationships among faecal egg count, anti-nematode antibody level and live weight in Angus cattle. Animal Science 76:167-174.

Neuenschwander TF-O, Miglior F, Jamrozik J, Berke O, Kelton DF, Schaeffer LR. 2012. Genetic parameters for producer-recorded health data in Canadian Holstein cattle. Animal 6:571-578.

Nobre PRC, Misztal I, Tsuruta S, Bertrand JK, Silva LOC, Lopes PS. 2003. Genetic evaluation of growth in Nellore cattle by multiple trait and random regression models. Journal of Animal Science 81:927-932.

Nødtvedt A, Dohoo I, Sanchez J, Conboy G, DesCjteaux L, Keefe G, Leslie K, Campbell J. 2002. The use of negative binomial modelling in a longitudinal study of gastrointestinal parasite burdens in Canadian dairy cows. Canadian Journal of Veterinary Research 66:249-257.

Pant SD, Verschoor CP, Schenkel FS, You Q, Kelton DF, Karrow NA. 2011. Bovine *PGLYRP1* polymorphisms and their association with resistance to *Mycobacterium avium* spp. *paratuberculosis*. Animal Genetics 42:354-360.

Pasandideh M, Mohammadabadi MR, Esmailizadeh AK, Tarang A. 2015. Association of bovine *PPARGC1A* and *OPN* genes with milk production and composition in Holstein cattle. Czech Journal of Animal Science 60:97-104.

Perri AF, Mejía ME, Licoff N, Lazaro L, Miglierina M, Ornstein A, Becu-Villalobos D, Lacau-Mengido IM. 2011. Gastrointestinal parasites presence during peripartum decreases total milk production in grazing dairy Holstein cows. Veterinary Parasitology 178:311-318.

Phiri IK, Phiri AM, Harrison IJS. 2007. The serum glucose and ß-hydroxybutyrate levels in sheep with experimental *Fasciola hepatica* and *Fasciola gigantica* infection. Veterinary Parasitology 143:287-293.

Purfield DC, Evans RD, Berry DP. 2019. Reaffirmation of known major genes associated with carcass-related metrics based on whole genome sequence within a large multi-breed cattle population. BMC Genomics 20:720.

Rajala-Schultz PJ, Saville WJA, Frazer GS, Wittum TE. 2001. Asscoiation between milk urea nitrogen and feritlity in Ohio Dairy cows. Journal of Dairy Science 84:482-489.

Rashidi H. 2016. Breeding against infectious diseases in animals. Dissertation, Wageningen.

Raue K, Tappe J, Hunsche C, Blazejak K, Jordan D, Lindenwald D, Waindok P, Raulk MK, Strube C. 2016. Der Einfluss einer Wiedervernässung von Weideflächen auf den Endoparasitenbefall von Rindern und Schafen. Tagung der Fachgruppe „Parasitologie und parasitäre Krankheiten“, Berlin, S.89.

Reist M, Erdin D, von Euw D, Tschuemperlin K, Leuenberger H, Chilliard Y, Hammon HM, Morel C, Philipona C, Zbinden Y, Kuenzi N, Blum JW. 2002. Estimation of energy balance at the individual and herd level using blood and milk traits in high-yielding dairy cows. Journal of Dairy Science 85:3314-3327.

Riggio V, Matika O, Pon-Wong R, Stear MJ, Bishop SC. 2013. Genome-wide association and regional heritability mapping to identify loci underlying variation in nematode resistance and body weight in Scottish Blackface lambs. Heredity 110:420-429.

Roy B, Brahma B, Ghosh S, Pankaj PK, Mandal G. 2011. Evaluation of milk urea concentration as useful indicator for dairy herd management: a review. Asian Journal of Animal and Veterinary Advances 6:1-19.

Rubio-Aparicio M, López-López JA, Sánchez-Meca J, Martín-Martínez F, Viechtbauer W, Van den Noortgate W. 2018. Estimation of an overall standardized mean difference in random-effects meta-analysis if the distribution of random effects departs from normal. Research Synthesis Methods 9:489-503.

Ruiz-Campillo MT, Molina Hernandez V, Escamilla A, Stevenson M, Perez J, Martinez-Moreno A, Donnelly S, Dalton JP and Cwiklinski K. 2017. Immune signatures of pathogenesis in the peritoneal compartment during early infection of sheep with *Fasciola hepatica*. Scientific Reports 7:2782.

Schöpke K, Gomez A, Dunbar KA, Swalve HH, Döpfer D. 2015. Investigating the genetic background of bovine digital dermatitis using improved definitions of clinical status. Journal of Dairy Science 98:8164-8174.

Schwarz D, Lefebvre DM, van den Bijgaart H, Davière J-B, van der Linde R, Kold-Christensen S. 2015. Global experience on ketosis screening by FTIR technology. Proc. Technical Meeting of the International Committee for Animal Recording (ICAR), Krakow, Poland, ICAR, Rime, Italy (2015), pp. 5-10, ICAR technical series no. 19.

Schwensow N, Fietz J, Dausmann KH, Sommer S. 2007. Neutral versus adaptive genetic variation in parasite resistance: importance of major histocompatibility complex suptertypes in a free-ranging primate. Heredity 99:265-277.

Silva MVGB, Van Tassell CP, Sonstegard TS, Cobuci JA, Gasbarre LC. 2012. Box-Cox transformation and random regression models for fecal egg count data. Frontiers in Genetics 2:112.

Streit M, Thaller G, Bennewitz J. 2015. Untersuchungen zur Umweltsensitivität der Milchmerkmale bei Deutschen Holstein (Dissertation). Züchtungskunde 87:37-46.

Swart GWM. 2002. Activated leukocyte cell adhesion molecule (CD166/ALCAM): Developmental and mechanistic aspects of cell clustering and cell migration. European Journal of Cell Biology 81:313-321.

Tao W, Mallard B, Karrow N, Bridle B. 2003. Construction and application of a bovine immune-endocrine cDNA microarray. Veterinary Immunology and Immunopathology 101:1-17.

Thompson-Crispi KA, Sewalem A, Miglior F, Mallard BA. 2012. Genetic parameters of adaptive immune response traits in Canadian Holsteins. Journal of Dairy Science 95:401-409.

Tilquin P. 2003. Methodological aspects of the mapping of disease resistance in livestock. Louvain-la-Neuve, Belgiuem: Université catholique de Louvain, PhD thesis.

Togersen PR, Schnyder M, Hertzberg H. 2005. Detection of anthelmintic resistance: a comparison of mathematical techniques. Veterinary Parasitology 128:291-298.

Twomey AJ, Graham DA, Doherty ML, Blom A, Berry DP. 2018a. Little genetic variability in resilience among cattle exists for a range of performance traits across herds in Ireland differing in *Fasciola hepatica* prevalence. Journal of Animal Science 96:2099-2112.

Twomey AJ, Carroll RI, Doherty ML, Byrne N, Graham DA, Sayers RG, Blom A, Berry DP. 2018b. Genetic correlations between endo-parasite phenotypes and economically important traits in dairy and beef cattle. Journal of Animal Science 96:407-421.

Twomey AJ, Berry DP, Evans RD, Doherty ML, Graham DA, Purfield DC. 2019. Genome-wide association study of endo-parasite phenotypes using imputed whole-genome sequence data in dairy and beef cattle. Genetic Selection Evolution 51:15.

Wagner M, Bilinska M, Pokryszko-Dragan A, Sobczynski M, Cyrul M, Kusnierczyk P, Jasek M. 2014. *ALCAM* and CD6 – multiple sclerosis risk factors. Journal of Neuroimmunology 276:98-103.

Westermann AJ, Gorski SA, Vogel J. 2012. Dual RNA-seq of pathogen and host. Nature Reviews Microbiology 10:618-630.

Williams SH, Mouchel N, Harris A. 2003. A comparative genomic analysis of the cow, pig, and human *CFTR* genes identifies potential intronic regulatory elements. Genomics 81:628-639.

Wit J, Gilleard JS. 2017. Resequencing helminth genomes for population and genetic studies. Trends in Parasitology 33:388-399.

Wittenburg D, Melzer N, Reinsch N. 2014. Genomic additive and dominance variance of milk performance traits. Journal of Animal Breeding and Genetics 132:3-8.

Zhao N, Chen J, Carroll IM, Ringel-Kulka T, Epstein MP, Zhou H, Zhou JJ, Ringel Y, Li H, Wu MC. 2015. Testing in microbiome-profiling studies with MiRKAT, the microbiome regression-based kernel association test. The American Journal of Human Genetics 96:797-807.

Zhou P, Du L-F, Lv G-Q, Yu X-M, Gu Y-L, Li J-P, Zhang C. 2011. Functional polymorphisms in *CD166/ALCAM* gene associated with increased risk for breast cancer in a Chinese population. Breast Cancer Research and Treatment 128:527-534.

Zintl A, Talavera S, Sacchi-Nestor C, Ryan M, Chryssafidis A, Mulcahy G. 2015. Comparison of *Fasciola hepatica* genotypes in relation to their ability to establish patent infections in the final host. Veterinary Parasitology 210:145-150.

Zwald NR, Weigel KA, Chang YM, Welper RD, Clay JS. 2004. Genetic selection for health traits using producer-recorded data. I. Incidence rates, heritability estimates, and sire breeding values. Journal of Dairy Science 87:4287-429.

ERKLÄRUNG

Erklärung gemäß der Promotionsordnung des Fachbereichs 09 vom 7. Juli 2004 § 17 (2)

„Ich erkläre: Ich habe die vorgelegte Dissertation selbstständig und ohne unerlaubte fremde Hilfe und nur mit den Hilfen angefertigt, die ich in der Dissertation angegeben habe.

Alle Textstellen, die wörtlich oder sinngemäß aus veröffentlichten Schriften entnommen sind, und alle Angaben, die auf mündlichen Auskünften beruhen, sind als solche kenntlich gemacht.

Bei den von mir durchgeführten und in der Dissertation erwähnten Untersuchungen habe ich die Grundsätze guter wissenschaftlicher Praxis, wie sie in der „Satzung der Justus-Liebig-Universität Gießen zur Sicherung guter wissenschaftlicher Praxis“ niedergelegt sind, eingehalten“.

Gießen, den 26.11.2019

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Katharina May