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Programa de Pós-graduação em Zootecnia

Gabriela Canabrava Gouveia

Estudo genético de características de resposta a parasitoses em bovinos criados em regiões tropicais

Belo Horizonte

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Tese apresentada ao Programa de Pós-Graduação em Zootecnia da Escola de Veterinária da Universidade Federal de Minas Gerais como requisito parcial para Obtenção do grau de Doutor em Zootecnia

Área de concentração: Genética e melhoramento animal

Prof. Orientador: Fabio Luiz Buranelo Toral

Coorientadores: Prof. Idalmo Garcia Pereira e Dr. Fernanda Santos Silva Raidan

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ESCOLA DE VETERINÁRIA DA UFMG
COLEGIADO DO PROGRAMA DE PÓS-GRADUAÇÃO EM ZOOTECNIA
Av. Antônio Carlos 6627 - CP 567 - CEP 30123-970 - Belo Horizonte - MG
TELEFONE (31) 3409 2173
www.vet.ufmg.br/academicos/pos_graduacao
E-mail: cpgzootec@vet.ufmg.br

ATA DE DEFESA DE TESE DE GABRIELA CANABRAVA GOUVEIA

Às 09:00 horas do dia 09 de abril de 2021, reuniu-se, remotamente, a Comissão Examinadora de Tese, indicada por ad referendum no dia 05/04/2021, para julgar, em exame final, a defesa da tese intitulada: **Estudo genético de resposta a parasitose em bovinos criados em regiões tropicais**, como requisito final para a obtenção do Grau de **Doutor em Zootecnia, área de concentração Genética e Melhoramento.** Abrindo a sessão, o Presidente da Comissão, Prof. Fabio Luiz Buranelo Toral, após dar a conhecer aos presentes o teor das Normas Regulamentares da Defesa de Tese, passou a palavra à candidata, para apresentação de seu trabalho. Seguiu-se a arguição pelos examinadores, com a respectiva defesa da candidata. Logo após, a Comissão se reuniu, sem a presença da candidata e do público, para julgamento da tese, tendo sido atribuídas as seguintes indicações:

	Aprovada	Reprovada
Dra. Luciana Correia de Almeida Regitano	X	
Profa. Laila Talarico Dias	X	
Dr. Tiago Luciano Passafaro	X	
Profa. Maria Raquel Santos Carvalho	X	
Prof. Fabio Luiz Buranelo Toral	X	

Pelas indicações, a candidata foi considerada: [X] Aprovada [ ] Reprovada

Para concluir o Doutorado, a candidata deverá entregar 03 volumes encadernados da versão final da tese acatando, se houver, as modificações sugeridas pela banca, e a comprovação de submissão de pelo menos um artigo científico em periódico recomendado pelo Colegiado dos Cursos. Para tanto terá o prazo máximo de 60 dias a contar da data defesa.

O resultado final, foi comunicado publicamente à candidata pelo Presidente da Comissão. Nada mais havendo a tratar, o Presidente encerrou a reunião e lavrou a presente ata, que será assinada por todos os membros participantes da Comissão Examinadora e encaminhada juntamente com um exemplar da tese apresentada para defesa.

Belo Horizonte, 09 de abril de 2021.

Assinatura dos membros da banca:

Tiago Luciano Passafaro

(Vide Normas Regulamentares da defesa de Tese no verso)
(Este documento não terá validade sem assinatura e carimbo do Coordenador)

À ciência e à produção animal, dedico.

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“O conhecimento é como uma escada:
quanto mais alto você sobe, mais ampla é sua visão.”
Sam Pitroda

RESUMO

As parasitoses causam prejuízos significativos na atividade pecuária e para diferentes espécies de interesse produtivo. Em regiões de clima tropical, em especial, as parasitoses são endemias e os impactos causados por elas são ainda mais expressivos. Isso acontece pois nessas regiões as condições edafoclimáticas propiciam a proliferação e disseminação de parasitas na fase infectante. O material apresentado a seguir tem como objetivo demonstrar a importância das principais parasitoses sobre a bovinocultura de corte e caracterizar geneticamente a resistência e a resiliência a parasitos, apresentando os diferentes mecanismos biológicos pelos quais os animais podem responder à essas parasitoses. Serão apresentados dois estudos: o primeiro deles buscou caracterizar geneticamente a resiliência a carrapatos, nematoides gastrointestinais e coccidioses em uma população de bovinos de corte da raça Nelore, em diferentes faixas etárias; o segundo teve como objetivo estudar as diferenças observadas na resistência a carrapatos em duas populações de bovinos, uma de animais da raça Nelore criados no Brasil e outra de animais Tropical Composite criados na Austrália. Com o presente estudo demonstramos que há variabilidade genética para as duas características abordadas: resistência e resiliência, mesmo para a população de animais Nelore em estudo, em que as contagens de parasitos observadas foram baixas. Ainda, resultados iniciais demonstram que os mecanismos biológicos associados à resistência nas populações de Nelore e Tropical Composite são parcialmente distintos entre si, e que possivelmente a composição racial (*Bos taurus* x *Bos indicus*) interfere na manifestação desse mecanismo de resposta à parasitose. Em relação a resiliência a parasitos, verificamos que os mecanismos biológicos associados a esse mecanismo de resposta se alteram em função da idade dos indivíduos (o que provavelmente está associado ao processo de amadurecimento do sistema imune) e do tipo de parasito sendo estudado. Os resultados apresentados aqui abrem espaço para discussão sobre respostas imunes envolvidas nos diferentes fenótipos, nas diferenças observadas em função da idade dos animais, ou da composição racial (em relação às diferenças esperadas na resistência entre animais taurinos e zebuínos).

Palavras-chave: CARRAPATOS; *EIMERIA SPP.*; GENES CANDIDATOS FUNCIONAIS; NEMATÓDEOS GASTROINTESTINAIS; RESILIÊNCIA; RESISTÊNCIA

ABSTRACT

Parasites cause significant loss at livestock industry and for different species of interest. At tropical regions, specially, parasitosis are endemic and their impact on livestock are even more expressive. This phenomenon happens because in these tropical regions edaphoclimatic conditions favour both the proliferation and the dissemination of parasites in the infecting stage. The material presented here has the objective of demonstrate the importance of the main parasites that affect the beef cattle production system and genetic characterize both the resistance and the resilience to parasites, presenting the different biological mechanisms by which animals respond to the parasite burden. We will present here two studies: the first is a study focused on the differences observed at host resistance to ticks in two populations, one of Nellore cattle raised in Brazil and the other of Tropical Composite cattle raised in Australia; the second aims to genetic characterize host resilience to ticks, gastrointestinal nematodes, and coccidia at a Nellore population across ages. In this thesis we demonstrated that there is genetic variability for both studied traits: resistance and resilience, even for the Nellore population, in which the observed parasite burdens were low. Also, our preliminary results demonstrate that the biological mechanisms associated with resistance to ticks at both Nellore and Tropical Composite populations are partially distinct, and that possibly the racial composition (*Bos taurus* x *Bos indicus*) interferes in the expression of the studied mechanism of response to parasitosis. Regarding host resilience to different parasites, we verified that mechanisms associated to its expression differ according to both animal's age (which is probably associated with immune system maturation), and regarding the studied parasite. The results presented in this thesis open space to the discussion of immune mechanisms evolved in the different phenotypes, of the differences observed in the response to disease across ages or between breeds (mainly regarding differences that are expected in host resistance between zebu and indicine animals).

Keywords: *EIMERIA SPP.*; FUNCTIONAL CANDIDATE GENES; GASTROINTESTINAL NEMATODES; RESILIENCE; RESISTANCE; TICKS

Sumário

INTRODUÇÃO GERAL

Em regiões de clima tropical, as parasitoses são endemias que acarretam prejuízos econômicos em sistemas produtivos de diferentes espécies. O clima quente e úmido favorece a manutenção do ciclo de vida e a proliferação dos parasitos. Dentre os parasitos que mais influenciam na produtividade dos animais estão os carrapatos, nematódeos gastrointestinais e coccídeos.

As parasitoses são problemas que afetam os sistemas produtivos há muitos anos, e o método padrão para controle dos parasitos é o uso de substâncias químicas de forma profilática e para o controle dos surtos de infestação. O principal problema com essas práticas é o desenvolvimento de resistência dos parasitos aos principais princípios ativos comercializados. Como alternativa, a seleção para características de resposta a parasitose apresenta potencial em reduzir os prejuízos causados pelas altas infestações, reduzir os custos causados pelo uso de agrotóxicos e com o manejo sanitário dos rebanhos e ainda contornar o problema causado pela resistência aos princípios ativos disponíveis no mercado.

Diferentes tipos de resposta a doenças (ou fatores estressores) têm sido discutidos e caracterizados geneticamente na literatura. Ainda não existe um consenso sobre a definição dos principais mecanismos. A título de discussão nesta tese, serão utilizados os conceitos de resistência, tolerância e resiliência, em que a resiliência é considerada uma resposta conjunta de fatores relacionados a resistência e a tolerância.

O objetivo desta tese foi caracterizar geneticamente dois fenótipos de resposta às parasitoses: resistência e resiliência. Ainda, objetivou-se estudar o impacto de fatores inerentes aos animais (idade e composição racial) e que podem interferir na expressão dos fenótipos estudados. Os principais conceitos utilizados para a realização desse estudo, assim como a demonstração da relevância do assunto, estão apresentados no primeiro capítulo dessa tese. Em seguida, dois capítulos com estudos distintos sobre resposta a parasitose serão apresentados: um que objetiva caracterizar geneticamente a resiliência do hospedeiro às parasitoses em diferentes estágios da vida dos animais; e outro com o objetivo de discutir as principais diferenças observadas nos parâmetros genéticos e nos mecanismos genéticos associados à resistência a carrapatos em animais Nelore (zebuínos) criados no Brasil e animais Tropical Composite (zebuínos x taurinos) criados na Austrália.

CAPÍTULO 1 - REVISÃO DE LITERATURA

1.1. Impacto das parasitoses na bovinocultura em regiões tropicais

Ao redor do mundo as doenças infecciosas reduzem a performance produtiva, reprodutiva e a sobrevivência dos animais de produção, limitando consequentemente a sustentabilidade e a lucratividade do sistema produtivo (KNAP; DOESCHL-WILSON, 2020). Na bovinocultura de corte, em regiões de clima tropical e subtropical, as parasitoses são doenças endêmicas e estão dentre as que mais afetam a produtividade dos rebanhos (CORWIN, 1997; DE LA FUENTE et al., 2008). Segundo OLIVEIRA et al. (2017), 55,12% e 22,35% dos diagnósticos de parasitoses em bovinos realizados na região sul do Rio Grande do Sul entre 1978 e 2014 foram de tristeza parasitária bovina e parasitose gastrointestinal mista, respectivamente. Do total de animais avaliados, 20,93% tinham aptidão para produção de leite e eram criados em regime de semiconfinamento e 79,6% eram bovinos de corte criados extensivamente. Ainda, os animais avaliados eram em sua maioria taurinos ou cruzados (apenas 5,9% de animais zebuínos) (OLIVEIRA et al., 2017).

A tristeza parasitária bovina é um complexo de doenças transmitidas majoritariamente por carrapatos, a exemplo da babesiose e da anaplasmose. Além da capacidade em transmitir parasitoses de alta prevalência em bovinos, os efeitos diretos dos carrapatos sobre o hospedeiro, tais quais anemia, imunossupressão e estresse, também levam a uma redução na eficiência produtiva dos animais (DE LA FUENTE et al., 2008). As parasitoses gastrointestinais mistas também levam à redução na eficiência produtiva dos animais por causarem, principalmente, diarreia e desidratação. Segundo OLIVEIRA et al. (2017), as parasitoses gastrointestinais mistas foram causadas principalmente por agentes dos gêneros *Trichostrongylus, Cooperia, Ostertagia, Strongyloides, Nematodirus* e *Oesophagostomum* – todos estes nematoides.

Um outro agente causador de diarreias em bovinos são os protozoários *Eimeria* spp., causadores de coccidioses, que são considerados um dos parasitos gastrointestinais de maior dificuldade em se controlar (EKAWASTI et al., 2019). Os coccídeos também causam gastroenterites e as coccidioses, juntamente aos nematoides gastrointestinais e aos carrapatos, estão entre as parasitoses mais comuns que afetam bovinos ao redor do mundo, sendo de alta prevalência no Brasil (CARDIM et al., 2018).

Em geral, animais altamente infestados pelos parasitas supracitados apresentam anemia, redução do apetite; problemas nos processos de digestão e absorção de proteínas, redução da energia disponível para crescimento e desenvolvimento, desequilíbrio hidroeletrolítico como consequência das diarreias e queda na imunidade (HAWKINS, 1993). A perda anual causada pelos principais endo e

ectoparasitas que acometem o gado brasileiro foi estimada por Grisi et al. (2014) em aproximadamente 14 bilhões de dólares.

Esses gastos tendem a ser ainda maiores em regiões tropicais, onde o tratamento profilático dos animais é frequentemente realizado, a fim de reduzir a carga parasitária nos animais. Em estudo realizado com 872 fazendas de produção de leite distribuídas ao longo de 8 estados no Brazil, apenas 22% não realizaram tratamento profilático para as parasitoses (CRUVINEL et al., 2020). Embora os gastos sejam altos, em regiões altamente infestadas o tratamento dos animais é um investimento que resulta em efeitos benéficos relacionados à produtividade, incluindo aumento no ganho de peso, melhora na conversão alimentar, aumento na produção de leite, melhoras na performance reprodutiva, qualidade de carcaça e resposta imune, além da possível redução da morbidade e mortalidade (FRISCH; O'NEILL; KELLY, 2000; HAWKINS, 1993).

Além de acarretarem gastos ao sistema produtivo, o uso de medicamentos para o controle de parasitoses pode deixar resíduos na carne e no ambiente, e resultar na seleção de parasitos resistentes aos princípios químicos ativos presentes nas fórmulas (DEMELER et al., 2009; GRISI et al., 2014). Para evitar as consequências negativas do uso de produtos químicos para o tratamento de animais acometidos, o uso de metodologias auxiliares ao controle de parasitos – a fim de reduzir os impactos das parasitoses sobre o desempenho animal é recomendado, e a seleção de animais mais resistentes ou tolerantes tem sido proposta na literatura (BISHOP; WOOLLIAMS, 2014; ZVINOROVA et al., 2016).

1.2. Mecanismos de resposta a parasitoses

A capacidade de responder positivamente à parasitose tem se tornado um dos atributos mais desejáveis em rebanhos de bovinos de corte. Os animais possuem dois mecanismos para responder às parasitoses: resistência, que corresponde a capacidade em suprimir o estabelecimento dos parasitas e eliminar as cargas parasitárias, consequentemente interrompendo o ciclo de vida do parasito; e tolerância, que corresponde à habilidade em minimizar os efeitos negativos causados no organismo do animal em função da parasitose (MULDER; RASHIDI, 2017). As importâncias relativas da resistência e da tolerância para o estabelecimento da infecção irão depender, principalmente, da prevalência das parasitoses no rebanho (BISHOP, 2012). A prevalência, por sua vez, é definida em função da resistência média dos grupos e da pressão de infestação, ou seja, da intensidade da infestação ambiental (BISHOP, 2012). A resistência e a tolerância são mecanismos de defesa

capturados pela resiliência do hospedeiro à parasitose (KNAP; DOESCHL-WILSON, 2020; RÅBERG; GRAHAM; READ, 2009).

A resiliência é definida como a capacidade do animal em minimizar os efeitos causados por agentes estressores ou rapidamente retornar ao estágio pertencente antes da exposição ao distúrbio (BERGHOF; POPPE; MULDER, 2019). A resiliência a parasitas pode ser avaliada por meio da comparação da performance de indivíduos submetidos a ambientes com diferentes níveis de infestação por parasitos (KNAP; DOESCHL-WILSON, 2020). Outros conceitos também relacionados à resiliência são encontrados na literatura. Berghof et al. (2019) citam os principais deles como: robustez, sensibilidade ambiental, plasticidade, susceptibilidade, canalização genética, dentre outros. Para o estudo de resposta a parasitoses, entretanto, os conceitos de resistência, tolerância e resiliência serão utilizados no presente estudo.

1.3.Seleção para características de resposta a doença

Diferentes indicadores têm sido usados para avaliar geneticamente resistência, tolerância e resiliência. A contagem de parasitos é o indicador mais utilizado para se estimar resistência a parasitos (PASSAFARO et al., 2015; PRAYAGA et al., 2009; RIBEIRO et al., 2020; TURNER et al., 2011). Como a distribuição dessas contagens frequentemente não se aproxima da normalidade, seja por não ser possível se observar contagens negativas ou, principalmente, por haver uma incidência maior de contagens zero ou baixas, mas com presença de contagens muito altas para alguns indivíduos, normalmente as contagens de parasitos são transformadas para uma escala logarítmica, ou categorizadas em scores de infestação. Uma outra possibilidade é modelar dados de contagem utilizando modelos lineares generalizados, em que não é feita a transformação do fenótipo, mas assume-se distribuições não gaussianas a priori no modelo (PASSAFARO et al., 2021; TEMPELMAN, 1998).

As herdabilidades estimadas para resistência a diferentes parasitos variam bastante em função da raça estudada, da característica utilizada como indicador de resistência e do delineamento utilizado para realização das contagens. Herdabilidades de 0,11 a 0,12 foram estimadas para contagens de carrapatos em escala logarítmica em bovinos Nelore (PASSAFARO et al., 2021; RIBEIRO et al., 2020), 0,37 para a mesma característica em população multirracial de bovinos criados na Austrália (TURNER et al., 2010), e de 0,17 a 0,25 para bovinos Hereford e Braford criados no Brasil (BIEGELMEYER et al., 2017; CAVANI et al., 2020). Para contagens de ovos de nematódeos

gastrointestinais e oocistos de *Eimeria* spp., herdabilidades de 0,06 e 0,16 foram estimadas para bovinos da raça Nelore, respectivamente (RIBEIRO et al., 2020).

Ainda, tem-se atribuído às baixas herdabilidades observadas para contagem de parasitos a alguns fatores: baixas cargas parasitárias, que podem representar desafio insuficiente para que a variabilidade para resistência seja de fato captada (FALCONER, 1990); uso de escores de contagem no lugar das contagens reais utilizadas (PRAYAGA et al., 2009; TURNER et al., 2010); e o uso de apenas uma medida pontual por animal, uma vez que são muitos os fatores ambientais de difícil controle que podem influenciar na contagem de parasitos, além de a contagem em si ser um processo altamente dependente do avaliador, levando à uma baixa repetibilidade dos resultados observados em um mesmo animal (GIGLIOTI et al., 2018). É importante salientar que o desenvolvimento de novas metodologias de coletas de dados e o uso de outros fenótipos indicadores de resiliência, a exemplo de reações de hipersensibilidade à picada na pele, parâmetros de resposta imune no sangue, estudos caso-controle, dentre outros, para a realização de estudos mais aprofundados ainda é recomendado (BURROW et al., 2019).

Vale ressaltar que, para alguns parasitos e em algumas raças, herdabilidades de moderadas a altas foram estimadas para contagens de parasitos (para se inferir sobre a resistência à parasitos). Nesse aspecto, ainda há muito o que se discutir sobre os principais mecanismos biológicos que influenciam a contagem a parasitos em diferentes populações, grupos genéticos, dentre outros. Avaliações genéticas para tolerância e resiliência, por sua vez, são menos frequentes. Esses fenótipos são de determinação mais difícil. Modelos de regressão aleatória são utilizados para determinar tanto tolerância quanto resiliência. O que difere as duas características é a forma em que o gradiente ambiental é definido. Enquanto a tolerância determinada pelo coeficiente angular da norma de reação quando um indicador de infestação ou infecção observados no animal são os utilizados, para se estimar a resiliência a infestação ou infecção do ambiente é utilizada para definir o gradiente ambiental (KNAP; DOESCHL-WILSON, 2020).

A maior parte dos estudos delineados para estimar a tolerância a fatores estressores em bovinos é voltada para o estudo da tolerância ao calor em bovinos de leite (BAENA et al., 2019; NGUYEN et al., 2016; OTTO et al., 2019). Em relação ao estresse térmico, a tolerância já foi avaliada pelo slope da norma de reação quando uma característica adaptativa ou de produção é avaliada em relação a temperatura retal em animais submetidos à diferentes níveis de estresse térmico (DIKMEN et al., 2013; OTTO et al., 2019). A resiliência ao estresse térmico também já foi avaliada. Nesse caso os

autores utilizaram o índice de temperatura x umidade para determinar o gradiente ambiental para o estudo da resiliência (NGUYEN et al., 2016).

Em relação a parasitoses, Twomey et al. (2018) estudaram resiliência de bovinos à *Fasciola hepatica* por meio de modelos de norma de reação, utilizando diferentes fenótipos de interesse (características relacionadas à fertilidade, produção de leite e carcaça) em função da prevalência de parasitos em diferentes combinações de rebanho e ano. Os autores verificaram que a variabilidade genética para resiliência é baixa, independentemente de qual fenótipo foi utilizado como variável dependente para se estimar a resiliência.

Acredita-se que alguns fatores sejam responsáveis pelo menor número de estudos que visem caracterizar geneticamente a tolerância e a resiliência a diferentes parasitos. Dentre eles está o fato de que a estimativa de parâmetros genéticos importantes, como a herdabilidade, não são obtidos diretamente. Isso acontece porque a variância genética estimada para o coeficiente angular é apenas um componente genético da variabilidade genética estimada para o fenótipo adaptativo ou produtivo em estudo. Da mesma forma, as variâncias residuais são estimadas para o fenótipo adaptativo em estudo. Consequentemente, é inviável estimar a herdabilidade como uma razão entre as variâncias genéticas aditivas e residuais estimadas para o fenótipo.

Nguyen et al. (2016) derivaram a herdabilidade para resiliência utilizando a solução dos slopes para cada fêmea avaliada (valor genético para tolerância) como fenótipo. Um modelo touro foi utilizado, e os fenótipos de tolerância foram analisados em função de um intercepto e um slope (efeito do pai sobre o valor genético para tolerância das filhas). As variâncias do efeito touro foram estimadas desconsideram-se a estrutura de pedigree, e as herdabilidades para tolerância foram estimadas por esse modelo, multiplicando-se a variância de touro por quatro para obtenção das variâncias genéticas aditivas (NGUYEN et al., 2016). Tal abordagem, entretanto, não é de uso padrão nos estudos de tolerância publicados até então.

Mesmo com os desafios citados acima, estudos recentes demonstram o potencial em se selecionar para resiliência a doenças na produção animal (BERGHOF; POPPE; MULDER, 2019; KNAP; DOESCHL-WILSON, 2020). Os autores sugerem, inclusive, que valores econômicos para resiliência podem ser estimados com base na redução dos custos com mão de obra e com técnicas de manejo sanitário nas propriedades, fatores que normalmente não são contabilizados para a construção de indicadores econômicos para fenótipos adaptativos utilizados para se estimar resiliência (BERGHOF; POPPE; MULDER, 2019). Ainda, acredita-se que a falta de ferramentas para se medir ou estimar a

resiliência, que atualmente limita a inclusão dessa característica nos programas de melhoramento genético de bovinos, podem ser superadas pelo uso de informações provenientes de marcadores moleculares associados à resiliência a parasitoses (e a outras doenças de interesse) nos programas (KNAP; DOESCHL-WILSON, 2020).

1.4.Estudos de associação genômica ampla aplicados à resposta do hospedeiro à parasitoses

Estudos de associação genômica ampla são realizados em ciência animal com objetivo de identificar regiões do genoma relacionadas com a expressão de características de importância econômica para o sistema produtivo. A aplicação destes estudos para predizer aspectos relacionados à predisposição a parasitoses e identificação das bases biológicas envolvidas, visando desenvolvimento de novas estratégias de prevenção e tratamento das mesmas é tida como objetivo central na genética humana (BUSH; MOORE, 2012). Na pecuária, essa metodologia foi utilizada para o estudo de mecanismos associados à contagem de carrapatos (PORTO NETO et al., 2011a; PORTO NETO et al., 2011b; RIBEIRO et al., 2020) e parasitos gastrointestinais (BENAVIDES; SONSTEGARD; VAN TASSELL, 2016; COLTMAN et al., 2001; RIBEIRO et al., 2020). Ainda, foram estudados mecanismos de tolerância ou susceptibilidade à algumas doenças de interesse, como a doença de Johne's e a tuberculose bovina (FINLAY et al., 2012; MINOZZI et al., 2010; ZANELLA et al., 2011). Características como tolerância e resiliência a endo e ectoparasitos ainda não foram associadas a nenhuma região do genoma e não se sabe se há influência genética na expressão destas, tampouco se há variabilidade genética suficiente para incluí-las nos programas de seleção.

Atualmente, além de identificar regiões associadas de maneira significativa aos fenótipos, metodologias estatísticas complementares foram desenvolvidas para se selecionar, dentre as regiões significativamente associadas ao fenótipo, quais os genes podem justificar a associação observada. A inclusão dessas metodologias de forma complementar aos achados dos estudos de associação genômica ampla é uma ferramenta fundamental por dois motivos: primeiro porque permite selecionar, com base nas regiões significativamente associadas ao fenótipos, quais são os mecanismos biológicos mais relevantes para se explicar o fenótipo expresso (SUBRAMANIAN et al., 2005); em segundo lugar porque permite filtrar, dentre os muitos genes associados a regiões de efeito significativo, quais de fato possuem capacidade em influenciar o fenótipo medido, e quais foram associados em reflexo ao alto desequilíbrio de ligação entre esses e os marcadores significativamente associados aos fenótipos nos estudos de associação genômica ampla (CHEN et al.,

2009). Essa utilização de metodologias complementares aos estudos de associação genômica ampla é o que deu início ao que chamamos era pós genômica (GALLAGHER; CHEN-PLOTKIN, 2018).

Análises de enriquecimento funcional são ferramentas utilizadas para comparar genes associados a marcadores significativos identificados em estudos de associação genômica ampla com termos de gene ontology (GLASS; GIRVAN, 2014). Os termos de gene ontology formam um dicionário controlado pelo consórcio de gene ontology (ASHBURNER et al., 2000). O objetivo do consórcio é criar um vocabulário de forma flexível e dinâmica, e que está em evolução constante, em que os genes homólogos e sequências proteicas são organizadas em função do mecanismo biológico compartilhado entre eles (ASHBURNER et al., 2000; GENE ONTOLOGY CONSORTIUM, 2010). Dessa forma, com base em uma lista de genes associados a um fenótipo específico em bovinos, por exemplo, é possível descrever as principais funções biológicas associadas ao fenótipo em estudo. Logo, os resultados de análises de enriquecimento funcional compreendem uma lista de funções biológicas que descrevem os resultados dos estudos de associação genômica ampla obtidos previamente.

De maneira complementar, foram desenvolvidas ferramentas de priorização de genes candidatos (CHEN et al., 2009). Basicamente, essas ferramentas calculam o score de similaridade entre cada gene identificado pelo estudo de associação genômica ampla com as funções biológicas enriquecidas (ou seja, que descreveram de forma significativa o fenótipo estudado) que foram obtidas por meio das análises de enriquecimento funcional previamente descritas.

A utilização conjunta de estudos de associação genômica ampla com análises de enriquecimento funcional e de priorização de genes candidatos permite: filtrar quais os genes de fato explicam o fenótipo de interesse; e descrever mecanismos biológicos associados ao fenótipo em estudo, com o objetivo de compreender melhor sua expressão. Tais conhecimentos permitem, ainda, identificar pontos chave que podem ser usados para o desenvolvimento de tratamento de doenças, desenvolvimento de vacinas, ou modificações no ambiente que possam favorecer o fenótipo estudado.

1.5.Considerações gerais

As parasitoses são doenças endêmicas e prejudiciais à bovinocultura de corte, principalmente em países tropicais. Compreender os mecanismos pelos quais um hospedeiro pode responder à parasitose, e o impacto que esta resposta tem sobre características de interesse produtivo pode

auxiliar na seleção das características a serem consideradas como critérios e objetivos de seleção, bem como nas práticas de manejo empregadas nas propriedades núcleo de melhoramento genético.

Diferentes características podem ser estudadas como indicadores de resposta à parasitose. O estudo das bases genéticas responsáveis pela manifestação desses indicadores medidos em animais de produção, possui potencial para reduzir os custos atribuídos ao tratamento das mesmas e os prejuízos decorrentes da redução do desempenho zootécnico dos animais. Muitos esforços foram direcionados para compreender os mecanismos de resistência à parasitos em bovinos, embora poucos mecanismos em comum sejam observados nos diferentes estudos. Acredita-se que a baixa reprodutibilidade dos estudos seja consequência do uso de populações muito distintas geneticamente e pelas possíveis diferenças observadas nos mecanismos biológicos ativados para a expressão de uma mesma característica nas diferentes populações.

Na contramão da resistência, pouco se sabe sobre os mecanismos envolvidos na expressão de tolerância e resiliência às principais parasitoses que afetam bovinos. Estudos iniciais que foquem na estimativa de parâmetros genéticos e principais mecanismos biológicos associados a essas características podem ampliar os conhecimentos existentes sobre as parasitoses e sobre maneiras alternativas de controlá-las.

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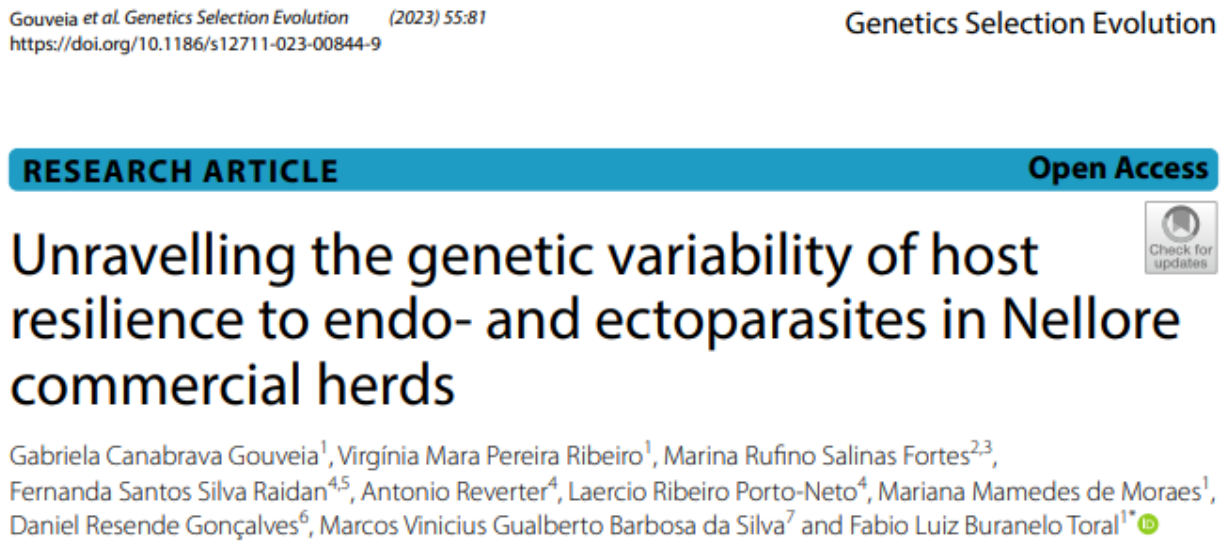
CAPÍTULO 2 - UNRAVELING THE GENETIC VARIABILITY OF HOST RESILIENCE TO ENDO AND ECTOPARASITES UNDER NATURAL INFESTATION, IN NELLORE CATTLE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Genetics Selection Evolution

RESEARCH ARTICLE Open Access

Unravelling the genetic variability of host resilience to endo- and ectoparasites in Nellore commercial herds

Gabriela Canabrava Gouveia[1], Virgínia Mara Pereira Ribeiro[1], Marina Rufino Salinas Fortes[2,3], Fernanda Santos Silva Raidan[4,5], Antonio Reverter[4], Laercio Ribeiro Porto-Neto[4], Mariana Mamedes de Moraes[1], Daniel Resende Gonçalves[6], Marcos Vinicius Gualberto Barbosa da Silva[7] and Fabio Luiz Buranelo Toral[1*]

Figure 2.1. Paper published at Genetic Selection Evolution in November, 2023

2.1. Abstract

Background:

Host resilience (HR) to parasites can affect the performance of animals. Therefore, the aim of this study was to present a detailed investigation of the genetic mechanisms of HR to ticks (TICK), gastrointestinal nematodes (GIN), and Eimeria spp. (EIM) in Nellore cattle that were raised under natural infestation and a prophylactic parasite control strategy. In our study, HR was defined as the slope coefficient of body weight (BW) when TICK, GIN, and EIM burdens were used as environmental gradients in random regression models. In total, 1712 animals were evaluated at five measurement events (ME) at an average age of 331, 385, 443, 498, and 555 days, which generated 7307 bodyweight (BW) records. Of the 1712 animals, 1075 genotyped animals were used in genome-wide association studies to identify genomic regions associated with HR.

Results:

Posterior means of the heritability estimates for BW ranged from 0.09 to 0.54 across parasites and ME. The single nucleotide polymorphism (SNP)-derived heritability for BW at each ME ranged from a low (0.09 at ME.331) to a moderate value (0.23 at ME.555). Those estimates show that genetic progress can be achieved for BW through selection. Both genetic and genomic associations between BW and HR to TICK, GIN, and EIM confirmed that parasite infestation impacted the performance of animals. Selection for BW under an environment with a con-trolled parasite burden is an alternative to improve both, BW and HR. There was no impact of age of measurement on the estimates of genetic variance for HR. Five quantitative trait loci (QTL) were associated with HR to EIM but none with HR to TICK and to GIN. These QTL contain genes that were previously shown to be associated with the production of antibody modulators and chemokines that are released in the intestinal epithelium.

Conclusions:

Selection for BW under natural infestation and controlled parasite burden, via prophylactic parasite control, contributes to the identification of animals that are resilient to nematodes and Eimeria ssp. Although we verified that sufficient genetic variation existed for HR, we did not find any genes associated with mechanisms that could justify the expression of HR to TICK and GIN.

Keywords: ticks, gastrointestinal nematodes, genetic parameters, *Eimeira* sp p., random regression models, response to disease, parasitic disease, GWAS

2.2. Background

Ecto- and endoparasites such as ticks (TICK), gastrointestinal nematodes (GIN), and Eimeria spp. (EIM) are endemic in tropical countries and responsible for several economic and productivity losses in cattle production systems [1]. Parasitic loads represent an important challenge for cattle

production, especially in tropical countries, such as Brazil. An animal's ability to respond to parasite loads is one of the stress factors that can impact the sustainability of the production system.

In the literature, different phenotypes are used to describe response to disease, among which we would like to highlight resistance, tolerance and resilience [2]. Resistance is the ability of the host to fully resist to infection, i.e., the ability to prevent parasite infection [3]. Tolerance is often described as the changes in the host's fitness, once it is infected, with respect to the evolution of the internal pathogen burden [3]. In contrast, resilience (HR) can be measured as the ability of the host, once infected, to maintain its fitness regardless of the internal pathogen burden [2, 4–6]. As highlighted by Knap and Doeschl-Wilson [2], HR can be defined as a combination of both resistance and tolerance mechanisms. In summary, the main diference between the last two defnitions is whether the levels of internal pathogen burden are considered (tolerance) or not (resilience).

HR can be estimated as a continuous trait using reaction norm models of the host's performance on environmental stress factors [2]. Under the assumptions of the reaction norm model, different patterns of growth depending on parasite burden can be described. There is no study in the literature that has attempted to estimate the different relationships between these factors, and we have no strong indication of the most adequate assumption for this. Tus, assuming a simplistic linear relationship between growth and parasite burdens, the additive variance of a performance trait can be decomposed into three components: the intercept variance (i.e. the additive component of the variability in performance assuming the absence of stress factors), the slope variance (i.e. the HR), and the covariance between intercept and slope [7]. Therefore, when linear regressions are used, the genetic correlation between the intercept and slope coefficients quantifies the genetic association between performance and HR [8].

Reaction norm models have been used to estimate HR to Fasciola hepatica in Irish cattle [9] and resilience of Rainbow trouts to freshwater × seawater [10]. Furthermore, Mulder [11] showed that these models can be used in selection programs to improve response to selection for resilience. In our study, we estimated HR to TICK, GIN, and EIM using the host's body weight and parasite burdens in random regression models. Therefore, our aim was to estimate genetic parameters for both BW and HR to TICK, GIN, and EIM and to identify genomic regions associated with these phenotypes in Nellore cattle.

2.3. Methods

2.3.1. Data collection and edition

We used the data on Nellore bulls that were born between 2010 and 2016 and raised on the Mundo Novo commercial farm, which is located in Uberaba, Minas Gerais state, Brazil (19°24′33″S and 48°06′34″W, at an altitude of 840 m, with a Monsoon-infuenced humid subtropical climate or Cwa weather according to the Köppen scale). Te Ethics and Animal Experimentation Committee of the Universidade Federal de Minas Gerais approved the experiment and data collection (Protocol 255/2010). Detailed description about the farm and the herd are in Passafaro et al. [12]. Te bulls were raised on pasture, which comprised mainly (>80%) grass of the Uruchloa genus, with a stocking rate of approximately 0.98 animal unit per hectare. Animals had free access to mineral supplementation and clean water throughout the year. After weaning (210 days old on average), the males were evaluated in performance tests that lasted 294 days, and included 70 days of adaptation, to minimize potential nutritional and social stress, and 224 days of evaluation (Fig. 2.2). Animals that were evaluated together, in the same performance test, were raised under the same environmental

conditions, ate grass of the same quality, and were subjected to similar social, adaptive, and environmental challenges for at least the last 56 days before the measurement events (ME).

The bulls were weighed at six ME: at day 1 of the performance test (data not used in our study), at the end of the adaptation period (day 70) and at four intervals of 56 days until the end of the test (Fig. 2.2), which defined five ME. The average age of the animals was 331, 385, 443, 498, or 555 days from the first to the fifth ME, respectively.

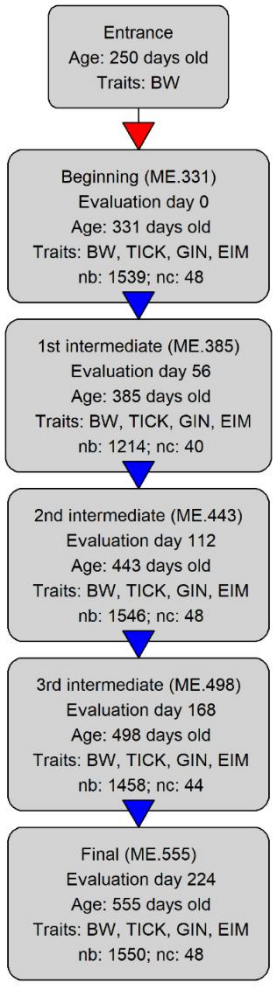

Figure 2.2. Diagram explaining data collection for performance tests of pasture raised cattle on the Mundo Novo farm—Brazil. Body weight (BW), ticks (TICK), eggs of gastrointestinal nematodes (GIN) and oocysts of Eimeria spp. (EIM) counts were collected at each measurement event (ME). "Age" represents the average age of animals at each ME. "nb" is the number of bulls and "nc" is the number of cohorts evaluated at each ME. Red arrow indicates a 70-day interval between evaluations, while blue arrows indicate a 56-day interval.

The tick counts used in the present study were obtained at each ME by counting the engorged female ticks, with a length size >4.5mm, on the right side of each animal [13]. The egg counts of gastrointestinal nematodes (GIN) and the oocyst counts of Eimeria spp. (EIM) were estimated by the number of eggs or oocysts per g of faeces, according to the modified McMaster technique [14].

Faecal samples were collected directly from the animals' rectum using properly identified and lubricated plastic bags. They were then cooled and transferred into chilled coolers in the laboratory. To perform the counts, we diluted 2 g of faeces with 28 mL of water, prepared 2-mL aliquots of this mixture and mixed each aliquot with 2 mL of saturated Sheater's solution (500 g of sugar, 6.5 mL of phenol and 360 mL of water). Then, a McMaster chamber was filled with 0.15 mL of the final solution to perform the counts of eggs and oocysts. Tus, in this study, tick, egg, and oocyst counts are the real counts observed on the right side of each animal or in the McMaster chamber.

Cohorts were defined by the combination of contemporary group (i.e. animals evaluated at a same performance test) and ME and only cohorts with more than five individuals were considered, thus, 7307 body weight records and parasite counts on 1712 animals were evaluated in this study (see Additional file 2.1: Table S1 and Additional file 2.2: Fig. S1). The pedigree file included 5944 animals from approximately nine generations, with 130 sires (with an average of 13.17 ± 12.46

offspring) and 1132 cows (with an average of 1.51 ± 0.77 offspring). The number of generations in the pedigree (generation coefficient) was calculated as follows:

$$GC_i = \left(\frac{GCS_i + GCD_i}{2}\right) + 1,$$

where GC_i is the generation coefficient of the individual *i*; GCS_i is the generation coefficient of the sire of animal *i*; and GCD_i is the generation coefficient of the dam of animal *i* [15]. Individuals with no known parent have a GC equal to one, which means that they belong to the base population. In the present study, the population had an average generation coefficient ± standard deviation of 5.55 ± 2.45, ranging from 1 to 9.81. The summary statistics for the data used in the present study are presented at Table 2.1

Table 2.1. Summary statistics for age at weighing (age), body weight (BW), tick (TICK), gastrointestinal nematode (GIN), and Eimeria spp. (EIM) counts at five measurement events (ME) in Nellore bulls

Trait	n	mean	sd	Median	Min	max
			ME.331			
Age (days)	1539	330.72	23.49	334.00	275.00	373.00
BW (Kg)	1539	223.08	33.23	220.00	138.00	343.00
TICK	1539	5.32	6.65	3.00	0.00	80.00
GIN	1539	4.71	6.82	2.00	0.00	80.00
EIM	1539	3.99	9.55	0.00	0.00	153.00
			ME.385			
Age (days)	1214	385.66	23.60	388.00	339.00	428.00
BW (Kg)	1214	238.87	35.44	237.00	135.00	411.00
TICK	1214	9.09	11.34	5.00	0.00	131.00
GIN	1214	4.93	6.33	3.00	0.00	43.00
EIM	1214	4.50	14.18	0.00	0.00	255.00
			ME.443			
Age (days)	1546	443.18	23.69	446.00	390.00	485.00
BW (Kg)	1546	261.21	36.29	260.00	156.00	380.00
TICK	1546	5.34	7.36	3.00	0.00	63.00
GIN	1546	5.79	7.64	3.00	0.00	80.00
EIM	1546	3.45	13.27	0.00	0.00	284.00
			ME.498			
Age (days)	1458	498.23	23.76	501.00	446.00	541.00
BW (Kg)	1458	305.67	36.84	306.00	176.00	429.00
TICK	1458	6.24	8.27	3.00	0.00	80.00
GIN	1458	5.13	6.28	3.00	0.00	71.00
EIM	1458	3.63	12.79	0.00	0.00	182.00
			ME.555			

Age (days)	1550	555.22	23.52	558.00	501.00	597.00
BW (Kg)	1550	337.27	37.91	336.00	214.00	467.00
TICK	1550	6.48	8.71	3.00	0.00	72.00
GIN	1550	4.22	6.20	2.00	0.00	73.00
EIM	1550	3.30	13.91	0.00	0.00	328.00

[1] n = number of observations; sd = standard deviation; min = minimum value; max = maximum value.

The bulls included in the present study were subjected to natural parasite infestation. Prophylactic parasite control is a routine strategy on the farm and integrates a group of sanitary management practices. In the studied herd, this strategy includes deworming with Ivermectin 4% (1 mL of Ivermectin per 50 kg of live BW—Master LP, Ouro Fino Saúde Animal, Cravinhos, SP) at the beginning of the performance tests (day 1 of the adaptation period). Approximately 65% of the bulls were dewormed. The choice of animals that received treatment was based on contemporary groups in such a way that all the animals that belonged to randomly chosen contemporary groups were dewormed. Blood samples were collected with sterilized syringes into 3.5-mL vacuum tubes containing 9NC coagulation sodium citrate 3.2%, to prevent blood from clotting and maintain DNA integrity. Blood samples were frozen and transferred into chilled coolers in the laboratory and stored in freezers at −20 °C. In total, 1230 blood samples were selected for genotyping with a low-density DNA array, i.e. the Z-chip v2 (Neogen, Lincoln, Nebraska, EUA, which contains 27,533 single nucleotide polymorphisms (SNPs) mapped to the ARSUCD1.2 bovine genome assembly). Most of the genotyped bulls were from the performance tests with more than 20 animals per group, as described above, and each animal had data for the three parasites for at least four ME. The quality control of DNA samples and markers was carried out using the SNP & Variation Suite v8.8.3 software [16]. Alleles with a GenTrain Score < 0.6 were considered as missing calls in the panel. Only SNPs with a call rate≥0.95, a minor allele frequency ≥ 0.05, and located on the autosomes and the X chromosome, and samples with a call rate > 0.90 were kept for further analyses. After quality

control, the SNP panel included 21,667 SNPs (78.7% of all tested SNPs) and 1075 samples (87.4% of genotyped samples).

2.3.2. Covariance components

We used single-trait linear random regression models (STM) where BW at each ME was considered as a dependent variable (trait) and the median count of the parasites for each cohort (Table 2.2) as an independent variable and no effect of co-infection, therefore we generated 15 datasets with both BW records and parasite counts. In the remainder of this paper, environmental parasite burden will refer to the median count of parasites for each cohort, which is our proxy for the strength of external (environment) infection, and not for individual parasite burden.

Table 2.2. Summary description of the median counts of ticks (TICK), gastrointestinal nematodes (GIN), and Eimeria spp. (EIM) per cohort

Trait per cohort	n	Median	Min	Max
ME.331				
TICK	48	3.50	0	16
GIN	48	3.25	0	9
EIM	48	0.00	0	11
ME.385				
TICK	40	7.00	0	33
GIN	40	4.00	0	11
EIM	40	2.00	0	10.50
ME.443				
TICK	48	2.75	0	15
GIN	48	4.00	0	12
EIM	48	0.00	0	16.50
ME.498				
TICK	44	4.00	0	16
GIN	44	3.00	1	8
EIM	44	0.00	0	11
ME.555				
TICK	48	4.25	0	18
GIN	48	2.75	0	8
EIM	48	0.00	0	7

The 15 STM were implemented using the Bayesian inference methodology and can be described as:

$$y_{ijkl} = C_j + d_1 m_{(k)} + b_0 + b_1 x_{(l)} + a_{0_{(i)}} + a_{1_{(i)}} x_{(l)} + e_{ijkl},$$

where y_{ijkl} is the weight of the animal *i*, evaluated for cohort *j*, at age *k* and submitted to an environmental parasite burden *l*; c_j is the systematic effect of cohort *j*; d_1, is the slope to fit the effect of age at which each animal was evaluated; $m_{(k)}$ is the age (in days) of the animals on the day of evaluation; b_0 and b_1 are the intercept and slope to fit the BW mean trajectory along the parasite burden, respectively; $x_{(l)}$ is the median of parasite counts (TICK or GIN or EIM) of the animals' cohort; $a_{0(i)}$ and $a_{1(i)}$ are the random intercept and slope to fit the additive genetic effect of each animal *i*, respectively; and e_{ijkl}, represents the error associated with each observation. It is important to highlight that $a_{0(i)}$ estimates the genetic effects for BW in the absence of parasite challenge, and $a_{1(i)}$ is

the HR. Furthermore, we assumed that there is a covariance between $a_{0(i)}$ and $a_{1(i)}$ and the variance components associated with those effects were estimated using pedigree information (traditional BLUP).

The additive genetic variances for BW for each observed environmental burden were estimated by the product P $\otimes$ G0 $\otimes$ P′. The P matrix has a number of lines equal to the number of different environments and two columns. The first column of P is a vector of 1s for adjusting the intercept, and the second column is the vector containing the observed median of parasite burden in the different cohorts; P′ is the transpose of the P matrix; G0 is the covariance matrix between the regression coefficients $a_{0(i)}$ and $a_{1(i)}$, and $\otimes$ is the Kronecker product. For further information about the STM see Additional file 2.3: Methods.

2.3.3. Genome-wide association studies

The 20 (4 traits×5 ME) genome-wide association studies (GWAS) were carried out for HR to the three parasites and for BW and five ME using the SNP & Variation Suite v8.8.3 software [16]. A mixed model was used to estimate the solutions for each of the 21,667 SNPs that passed quality control and an association test P-value related to each SNP solution was generated. For the GWAS, we used genotypes from animals for which their samples passed quality control (1075 samples). For all these animals, both BW records and estimated breeding values (EBV) for HR were available. The GWAS model used for BW can be described as:

$$BW = Xb + Zu + Sa + e;$$

where BW is the vector of body weight records for each animal at each evaluated age; X is the incidence matrix for the fixed covariates (cohort and age); b is the vector of solutions for the fixed effects; Z is an incidence matrix for the genetic additive random effects (estimated from the GRM); u is the vector of the solutions for the random additive genetic effects related to the observations; S is a matrix of genotypes (coded as 0, 1, or 2 copies of minor allele) for the evaluated SNPs, with number of rows equal to the number of genotyped animals, and number of columns equal to the number of SNPs in the genotype panel; a is the estimated effect of the evaluated SNP; and e is the vector of errors associated with each observation. SNP & Variation Suite v8.8.3 [16] uses a restricted maximum likelihood to estimate the solutions for the unknown parameters of the model.

Estimated breeding values estimated for HR to each parasite at each ME using STM were considered as the pseudo-phenotypes of HR for GWAS with no additional fixed effect. Note that using EBV as phenotypes may lead to double-counting of information and heterogeneous residuals. Given that all animals in the analysis had own phenotypes, it is expected that they had reasonably similar EBV, and thus homogeneous residuals, and that the double-counting of information was limited. Tus, the model used for GWAS for HR can be described as:

$$HR = Zu + Sa + e;$$

where HR is the vector of EBV for host resilience to TICK, GIN, or EIM at each ME and the other terms are as previously described. The GRM used for GWAS was calculated according to the first method of VanRaden [17]. We applied it with a full dosage compensation correction to include the markers on the X chromosome in the calculation of the GRM since the number of copies of the alternate allele at any locus of X-chromosome can only be zero or one, which makes allele frequency calculations for SNPs at X-chromosomes in males different from the calculation of allele frequencies

at autosomes [18]. It is not our objective to discuss about the effect of dosage compensation on the evaluated traits. However, there is evidence of this effect on the variation of complex phenotypes [19], thus we proceeded with the dosage compensation correction. Further information about the topic is described in Sidorenko et al. [19].

It is important to highlight that, in the present study, the heritability for BW was estimated based on both the GRM and the pedigree-based relationship matrix, thus for BW we present both the conventional heritability and the SNP-derived heritability estimates.

The pairwise SNP correlations of BW with HR to TICK, HR to GIN, and HR to EIM for each ME were computed by the Pearson correlation between the SNP effects of each one of the traits, as proposed by Fortes et al. [20], and will be considered in the present paper as proxies for the genetic correlations between traits.

2.3.4. Quantitative trait locus associated with host resilience

The sample-size-based approach described by Willer et al. [21] was used to perform the meta-analysis to combine the results of the five described GWAS of HT to TICK (GIN and EIM) using the SNP & Variation Suite v8.8.3 [16]. In summary, a Z-score and an overall P-value for each marker were calculated by combining the SNP P-value, direction of the effect, and sample size generated by the previous GWAS. The meta-analysis was performed for markers that had solutions estimated in at least two studies (i.e. for at least two ME) and no genomic control was performed during the meta-analyses. Then, we used a Bonferroni correction to define the two groups of SNPs: those that were significantly associated with a P-value$< 10^{-4}$ [22, 23].

Based on the results of the meta-analysis, we defined quantitative trait loci (QTL) associated with each trait. The QTL boundaries were defined as follows: first, we identified an initial peak, i.e. the SNP with the lowest P-value for each chromosome (Chr); second, we searched for significant SNPs within 0.5-Mbp regions up and downstream of the peak SNP. If we identified other significative SNPs within this interval, the boundaries of the QTL were expanded to include the SNP and another 0.5-Mbp region (up and downstream) was investigated. The process was repeated until there was no more significant SNPs in these 0.5-Mbp windows. Finally, a new peak SNP was called if there was a significant SNP on the same Chr but outside of the boundaries of the first QTL. The process was repeated for each Chr until no more peak SNPs could be identified.

Moreover, only regions with at least four significant or suggestive SNPs were considered as QTL (adapted from van den Berg et al. [24]). In addition, only suggestive SNPs (P-value < 10^{-4}) that were in high linkage disequilibrium (LD) with the peak or another significant SNP in the QTL were considered. The LD between SNPs was evaluated by the D prime (D') value that was estimated using the expectation–maximization method by pairwise analysis in the SNP & Variation Suite v8.8.3 [16]. SNPs were considered in high LD when D′ was greater than the mean + 2 standard deviations of the D′ computed between all combinations of SNPs on the same Chr.

We searched for genes located within the QTL boundaries using the ARS-UCD1.2 bovine genome assembly (available at https://www.ncbi.nlm.nih.gov/assembly/ GCA_002263795.2) with the GALLO package [25] of the R software [26]. This process resulted in a list of target candidate genes for HR to each parasite, which were used for the candidate gene prioritization analysis.

Candidate gene prioritization analysis was conducted using the ToppGene Suite [27] and consisted of twosteps. First, for each trait, a functional enrichment analysis was performed by building a list of the

genes that were more likely to be related with our phenotypes, hereafter named the trained gene list. Tis trained gene list was constructed based on keywords (see Additional file 2.1: Table S2) that describe each of the evaluated phenotypes (BW and HR to the three parasites). These lists were obtained using the web application GUILDify v2.0 [28] for the phenotypic characterization of genes. GUILDify searches for genes starting from user-provided keywords in the Biologic Interaction and Network Analysis (BIANA) knowledge database. The genes associated with the keywords are used as seeds to generate the protein interaction networks, for the selected organism, and analysed with graph theory algorithms to prioritize new disease genes [28]. In the present study, the selected model organism was *Homo sapiens*, since bovine was not an option. The Netscore prioritization algorithm from the GUILD package was used (with repetition=3 and interaction=2; default values of GUILDify). The output of GUILDify is a trained list of genes that are ranked according to the interaction network. The first 100 genes were used as the trained gene list for each studied trait.

For functional enrichment analysis, the trained gene list was compared with random sets of genes in the genome to search for any functional category or parameter that was overrepresented in our trained list compared with the background. We used Gene Ontology (Molecular function, Biological process, and Cellular component), Human phenotype, Mouse phenotype, Pathway, PubMed, Transcription factor binding site, Co-expression, and Disease as training databases. The P-value cut-off for each training parameter was 0.05 with a false discovery rate correction. After this step, a representative profile of the trained gene list was obtained.

In the second step, a similarity score was generated for each gene in our list of candidate genes. This score is created by functional annotation of the candidate gene followed by a comparison of its function to each enriched term that is learned in the training step. The similarity score calculation and the associated P-values are described in Chen et al. [27]. In summary, a fuzzy-based similarity

measure is applied for categorical terms [29], and Pearson correlation between the test gene and the enriched gene lists is applied for quantitative functional parameters. In the case of a missing value (for instance, lack of one or more annotations for a test gene), the score is set to −1. Otherwise, it is a real value within [0, 1] [27]. At the end of this process, each gene will have one similarity score and one P-value for each one of the functional categories.

A final test is carried out to compute an overall *P-value* that will be used to determine if each gene was prioritized or not. For this computation, we need to assume that the *P-values* come from independent tests, thus the Fisher's inverse chi-square method was applied to combine the P-values from multiple annotations into an overall *P-value* [27]. The prioritized genes were considered to be those with an overall *P-value* ≤ 0.05. For the candidate gene prioritization analysis, we used the default setting in the ToppGene Suite that has a background gene set from the genome for computing the P-value with 5000 coding genes and two features to be considered for prioritization.

2.4. Results

2.4.1. Genetic parameters for body weight and host resilience

In general, the highest posterior density interval with 90% of samples (HPD90) related to the posterior mean of the intercept and slope variances were wide, indicating no difference between genetic parameters estimated across ME. However, residual variances of BW estimated at ME.331 were smaller than those at ME.555 (Table 2.3). The posterior means of the correlation between intercept and slope were negative, but the HPD90 associated to those estimates were wide and included zero (Table 2.3).

Table 2.3. Posterior means of genetic parameters (limits of HPD90) for intercept (int) and slope coefficients of body weight at five measurement events (ME) when ticks (TICK), gastrointestinal nematodes (GIN), and Eimeria spp. (EIM) burden[a] were used as independent variables in single-trait linear random regression models

ME[2]	σ^2_{int}	σ^2_{slope}	$r_{int\ x\ slope}$	σ^2_e
		TICK		
331	186.15 (108; 262.4)	1.31 (0.29; 2.19)	-0.9 (-1.00; -0.78)	360.51 (320.7; 401.6)
385	81.05 (9.92; 144.5)	0.32 (0.03; 0.59)	-0.29 (-0.92; 0.52)	465.09 (413.7; 518.3)
443	112.68 (27.18; 194.8)	0.95 (0.02; 1.84)	-0.54(-1.00; -0.01)	467.66 (416; 517.8)
498	145.38 (54.55; 231.1)	1.09 (0.11; 2.03)	-0.45 (-0.97; 0.03)	527.59 (465; 588.4)
555	126.41 (43.78; 214.2)	1.03 (0.06; 1.95)	0.02 (-0.65; 0.87)	535.91 (470.8; 599.8)
		GIN		
331	163.83 (70.57; 256)	4.53 (1.59; 7.67)	-0.66(-0.91; -0.45)	341.2 (297.8; 390.6)
385	188.58 (64.33; 301.4)	4.05 (0.97; 6.87)	-0.74(-0.94; -0.54)	439.23 (380.9; 496.9)
443	119.87 (17.18; 213.5)	1.68 (0.06; 3.1)	-0.53 (-1.00; 0.08)	468.77 (419.8; 519.4)
498	222.44 (8.12; 405.6)	6.86 (0.39; 12.92)	-0.60(-0.98; -0.17)	529.12 (468.7; 590.2)
555	69.36 (1.52; 130.1)	4.68 (0.56; 8.57)	0.18 (-0.50; 1.00)	549.7 (485.8; 610.4)
		EIM		
331	105.77 (38.78; 165.4)	2.65 (0.49; 4.51)	-0.33 (-0.83; 0.14)	341.27 (291.7; 385.2)
385	161.31 (57.58; 256.3)	8.16 (2.82; 13.5)	-0.75(-0.96; -0.53)	431.66 (375; 489.6)
443	74.85 (18.39; 131.3)	2.23 (0.42; 3.92)	-0.07 (-0.80; 0.72)	462.04 (412; 513.1)
498	101.27 (23.98; 172.6)	3.51 (0.62; 6.42)	-0.07 (-0.70; 0.57)	530.03 (470.8; 591.1)
555	192.4 (94.15; 292.5)	7.51 (0.96; 12.97)	-0.52(-0.92; -0.14)	536.81 (469.8; 605)

[1] $\boldsymbol{\sigma^2_{int}}$ = additive genetic variance for the intercept; $\boldsymbol{\sigma^2_{slope}}$ = additive genetic variance for the slope; $r_{int\ x\ slope}$ = genetic correlation between intercept and slope; $\boldsymbol{\sigma^2_e}$ = residual variance. ME: measurement events when the age of animals was 331, 385, 443, 498 and 555 days on average.

* Parasitic burden was modelled using information about the median infestation per cohort (contemporary group)

A rising trend for the additive variance and heritability of BW was observed across the trajectories of TICK, GIN, and EIM burden (Fig. 2.3). For instance, the posterior means for the heritability of BW ranged from 0.09 to 0.44 at ME.331, from 0.13 to 0.51 at ME.385, from 0.13 to 0.54 at ME.443, from 0.16 to 0.45 at ME.498 and from 0.11 to 0.42 at ME.555. In spite of the differences between the heritability estimates for BW when parasite count was zero and maximum (maximum count of 16 for TICK, 11 for GIN and 10.5 for EIM), the HPD90 related to these posterior means were large showing no significant differences between them (Fig. 2.3).

The SNP-derived heritability (average ± standard error) for BW (Table 2.4) at each ME ranged from a low (0.09 ± 0.06 at ME.331) to a moderate magnitude (0.23 ± 0.06 at ME.555), showing that genetic improvement of BW can be achieved through selection. Moreover, these values were similar to the heritability of BW estimated by STM (Fig. 2.3).

Table 2.4. SNP-derived heritability estimates (standard error) for body weight (BW) and host resilience to ticks (HR.TICK), gastrointestinal nematodes (HR.GIN) and Eimeria spp. (HR.EIM) at diferent measurement events (ME)

Trait	ME.331	ME.385	ME.443	ME.498	ME.555
BW	0.16 (0.06)	0.09 (0.05)	0.16 (0.05)	0.19 (0.06)	0.23 (0.06)
HR.TICK	0.81 (0.04)	0.87 (0.04)	0.81 (0.04)	0.87 (0.03)	0.76 (0.04)
HR.GIN	0.84 (0.04)	0.93 (0.03)	0.80 (0.04)	0.84 (0.04)	0.85 (0.04)
HR.EIM	0.79 (0.04)	0.82 (0.04)	0.77 (0.04)	0.80 (0.04)	0.84 (0.03)

ME.331, ME.385, ME.443, ME.498, ME.555 are the measurement events when animals' ages were 331, 385, 443, 498 and 555 days in average.

The SNP-derived heritability estimates for HR to TICK, GIN, and EIM at each ME were computed through GWAS when the slope solutions (genetic effects) were considered as the HR phenotype. As expected, the magnitude of these estimates was large (Table 2.4), ranging from 0.76 to 0.87 for HR to TICK, from 0.80 to 0.93 for HR to GIN, and from 0.77 to 0.84 for HR to EIM.

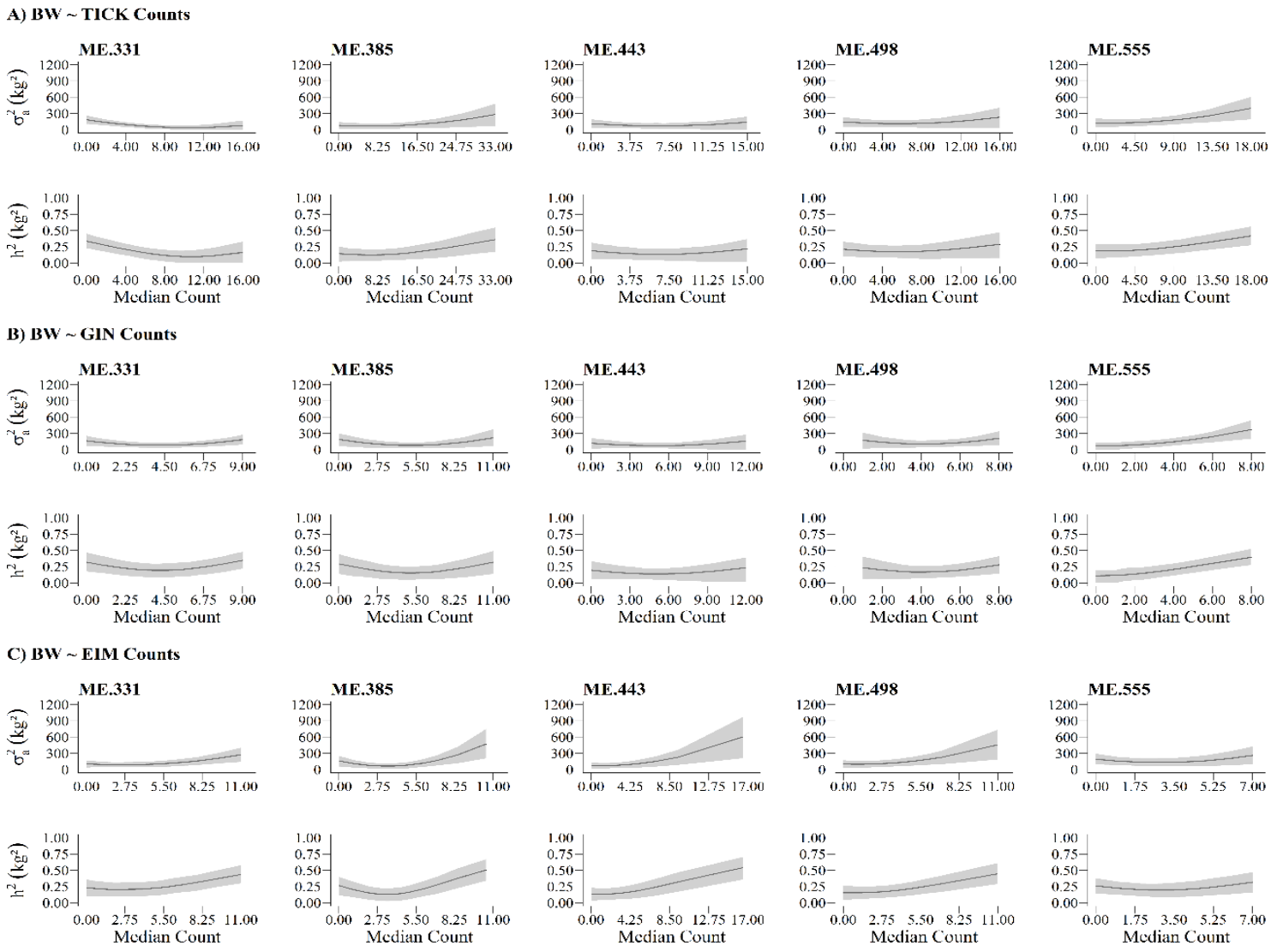

Figure 2.3. Additive genetic variances (σ_a^2, Kg^2) and heritability estimates (h^2) for body weight (BW) across the trajectories of tick (TICK), nematodes (GIN), or Eimeria ssp. (EIM) burden at five measurement events (ME). ME.331, ME.385, ME.443, ME.498, ME.555 are body weights at each measurement event when the average age of animals was 331, 385, 443, 498 and 555 days, respectively.

While in some cases, the genetic correlations between intercept and HR did not differ from zero (because HPD90 includes a zero value), the pairwise SNP correlations indicate that there is some genetic association between BW and HR. In general, the pairwise SNP correlations of BW with HR to GIN and HR to EIM were zero, or favorable (Fig. 2.4). However, pairwise SNP correlations of BW with HR to TICK at ME.331 (−0.648 ± 0.005), ME.443 (−0.307 ± 0.006), and ME.498 (−0.148 ± 0.007) were unfavorable (Fig. 2.4). These correlations agree with those estimated between intercept and slope, that were also negative (Table 2.3).

2.4.2. Candidate genes and pathways associated with host resilience to TICK, GIN, and EIM

The genes that were associated with HR were searched within the QTL that were built from the significant and suggestive SNPs obtained by the meta-analysis GWAS (Fig.2.5). The meta-analysis was processed using the GWAS results related to each age, separately, which are presented in Additional file 2.4: Fig. S2, Additional file 2.5: Fig. S3, and Additional file 2.6: Fig. S4. Information on the number of SNPs and linkage disequilibrium thresholds used to define the QTL boundaries are in Table 2.5.

Table 2.5. Description[1] of QTLs associated with host resilience to *Eimeria* spp.

CHR	N_{SNP}	N_{sigSNP}	N_{sugSNP}	LD_{CHR}	sd_{LD}	LD_{1-n}
4	11	1	3	0.17	0.19	0.70
6	14	2	2	0.15	0.16	0.51
7	11	3	1	0.15	0.16	0.85
12	17	3	1	0.17	0.17	0.71
13	39	2	6	0.15	0.16	0.54

[1]CHR = chromosome; N_{SNP}=number od SNPs inside QTL; $N_{peakSNP}$=number of significant SNPs inside QTL (associated *P-values* < $2.31x10^{-6}$); $N_{suppSNP}$=number of suggestive SNPs inside QTL (associated *P-values* > $2.31x10^{-6}$ and $<10^{-4}$); LD_{CHR}= average linkage disequilibrium observed between SNPs of each CHR; sd_{LD}=standard deviation of LD_{CHR}; LD_{1-n}= linkage disequilibrium between first and last SNP of QTL.

Apart from the presence of some significant isolated SNPs, we detected no QTL, i.e. no genomic regions, that were associated with HR to TICK and HR to GIN. Five QTL located on Chr 4, 6, 7, 12, 13 were associated with HR to EIM (Table 2.6). In total, 47 genes were located within these QTL regions (Table 2.6) and among these, 16 were prioritized. Information about the genes that were prioritized for HR to EIM is in Additional file 2.1: Table S3.

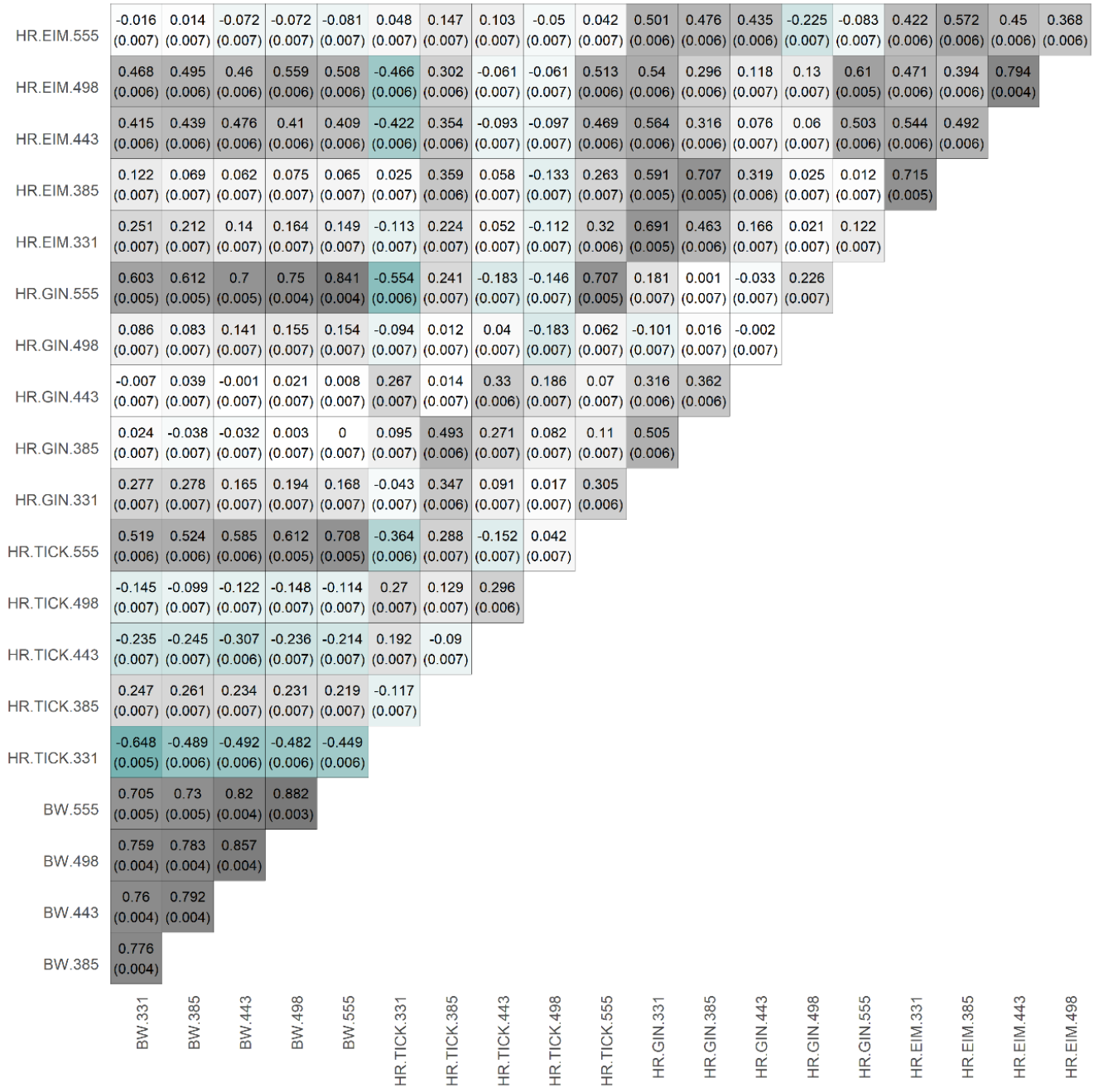

Figure 2.4. SNP correlations between body weight (BW), host resilience to ticks (HR.TICK), gastrointestinal nematodes (HR.GIN), and *Eimeria* spp. (HR.EIM) measured at five measurement events (ME - averaged animals' age was 331, 385, 443, 498, and 555 days old). The values above the diagonal are the Pearson correlations between SNP effects (and standard errors of SNP correlations).

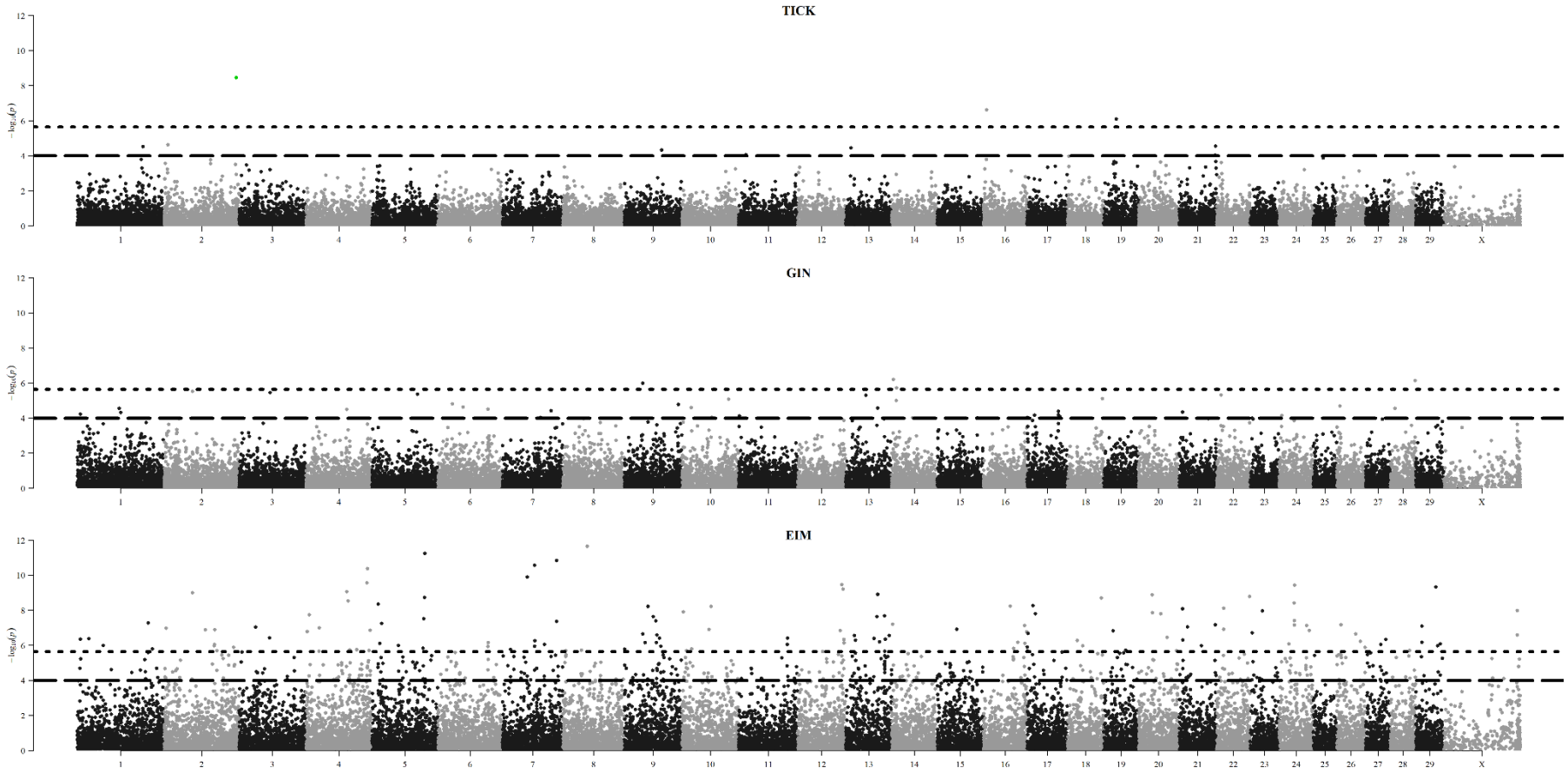

Figure 2.5. Manhattan plots for the meta-analysis of the genome-wide association studies for HR to ticks (TICK), gastrointestinal nematodes (GIN), and Eimeria spp. (EIM) measured at different measurement events. The dotted line (y=5.64) indicates the threshold for statistical significance. The dashed line (y=4.00) indicates the threshold for suggestive evidence of association.

Table 2.6. Description of the quantitative trait locus (QTL) defined from SNPs that were significantly associated with host resilience to *Eimeria spp*.

CHR	n	IP	FP	Genes inside QTL
4	11	116439784	117037674	DPP6[a] , HTR5A[a] , PAXIP[b] , RF00006[c]
6	14	90646323	91785192	CXCL9[a], CXCL10[a], CXCL11[a], NAAA[a], SCARB2[a], STBD1[a], ART3[b], CCDC158[b], NUP54[b], PPEF2[b], SDAD1[b], SHROOM3[b], SOWAHB[b], ENSBTAG00000004921[c], ENSBTAG00000032074[c], ENSBTAG00000050665[c], ENSBTAG00000053885[c], ENSBTAG00000054432[c], SEPT11[c], RF00003[c], RF00026[c]
7	11	58461990	59477630	DPYSL3[a], SPINK1[a], SPINK5[a], JAKMIP2[b], SCGB3A2[b], SPINK6[b], STK32A[b], bta-mir-2284y-7[c], C7H5orf46[c], ENSBTAG00000052309[c], ENSBTAG00000053960[c], RF00026[c]
12	17	83457070	84943864	COL4A1[a], IRS2[a], LIG4[a], TNFSF13B[a], ABHD13[b], MYO16[b], RF00001[c]
13	39	70341842	71369326	PTPRT[a], ENSBTAG00000002446[c], RF00026[c]

Genes marked with a were prioritized in the candidate gene prioritization analyses, b were included in the analyses but not prioritized, and genes with c were not included in the prioritization analyses

Chr chromosome, n number of SNPs within the QTL, IP initial position, FP final position

2.5. Discussion

2.5.1. Environmental parasite burden

In our study, the median counts of parasites were used as environmental gradient, once we considered it the best descriptor of parasitic load challenging a group of contemporary animals. First, it is important to mention that the environmental and animal loads are highly dependent due to the fact that life cycle of parasites involves a period of time spent in a host organism. For instance, the adult tick females lay their eggs in the pasture, where hatching occurs. Larvae, nymphs, and adults parasitize the host through feeding, and then drop of again on the pasture to continue their life cycle [4]. Similarly, gastrointestinal parasites and Eimeria spp. have multiple stages of development that include some time spent in the host organism but not their entire life cycle [30, 31]. However, individual loads depend on factors, such as animal resistance,

behavior, and other individual factors, and, thus, using individual parasitic loads can mask the real environmental challenges. For instance, more resistant animals might be within cohorts that are highly challenged and even then, present zero parasite counts. The opposite is also true, when exposed to low environmental loads, a highly susceptible animal might face a high parasitic load, although most of its contemporaries are parasite free.

HR, which is the main object of our study, is better estimated when environmental parasitic loads are available [32]. Such measurements were not available since we worked with data from a commercial herd, which means that the animals were not submitted to a high and artificially infested environment. Tus, we used the parasite counts observed in different animals from the same cohort as an indicator of the environmental load. Since counts are discrete measurements, we feel that using medians instead of average counts would better describe the common load to which all animals from a same cohort were exposed. It is important to highlight that we developed this study based on environmental parasitic burden, which is a proxy for environmental infection pressure (or strength of environmental infection), and not for host parasitic burden.

At least one animal in each cohort had parasite counts greater than zero, even in cohorts for which median parasite counts are zero, therefore there were no parasite free cohorts and every single animal in our dataset was exposed to natural infestation of TICK, GIN and EIM. A zero environmental load only indicates that the animals in these cohorts are less challenged than those in cohorts with a median parasite load equal to 5, for example. The observed median counts reported here are similar to those of other datasets in crossbreed Angus and Nellore [33], Colombian Bos taurus cattle breeds [34], and German Black and White dairy cows [35]. The low parasite load observed here might be partially explained by different factors, including the breed evaluated. Nellore cattle is an indicine breed known to be more resistant to highly infested environments [36, 37]. In addition, the adoption of rotational grazing [38], and prophylactic parasite control strategies largely applied in commercial herds might contribute to low parasite loads. It is important to have in mind that such controlled parasite burden through prophylactic treatment, as in this study, represents the reality on commercial farms [39–41].

2.5.2. Genetic parameters for body weight and host resilience

The SNP-derived and STM heritability estimates for BW obtained here were similar to those reported previously for the same population [42]. These results confirmed that the low-density SNP panel (27K—Z-chip V2, Neogen, Lincoln, Nebraska, EUA) can capture the polygenetic component of the additive variance observed for BW in Nellore cattle. The heritability estimates for BW in this population were lower than those reported in the literature for Nellore cattle of similar ages raised in Brazil, using pedigree information only [43], which can be partially explained by the fact that the studied population is under selection. Selective breeding can lead to lower genetic variability, and consequently, lower heritability estimates [44]. The selective breeding program from Mundo Novo farm was implemented in 1978 without including external candidates, with an intense selection for BW, and an efficient animal husbandry approach that corresponds to the outstanding practices of a nucleus farm. Regarding the high SNP-derived heritabilities estimated for HR in the present study, they do not indicate that HR is highly heritable. In fact, these values are a statistical artefact since the phenotype of HR is itself an estimated breeding value. However, the SNP-derived heritability indicates that breeding values estimated using pedigree-based genetic evaluations can be efficiently explained by the genomic similarity between individuals.

In the present study, we observed a curved trajectory of the heritability estimates for BW as the parasitic loads increased, with an overall rising trend if we compare extreme environments only, however this increase was not significant since the HPD90 overlapped. Marques et al. [45] observed a rising trend of the heritabilities for faecal egg counts (FEC) and a reduction in the heritabilities for BW in Corriedale sheep between environments with low or high FEC, but as in our case, these differences were not significant because the HPD of heritability estimates overlapped. Challenging environments with higher natural or artificial parasite loads are expected to lead to more significant effects on both BW and the genetic parameters for HR [46]. For instance, the highest heritability estimates for the FAMACHA score in ram and ewe lambs were obtained in high worm burden scenarios [47]. Similarly, an uprise in the trend of heritability estimates for milk yield was observed with increased temperature-humidity index, a direct indicator of heat stress [48]. However, it is important to highlight that

opposite trends for genetic variances and heritability estimates can be observed with increasing parasite burden. Hollema et al. [49] showed that a significant decrease in the heritability for growth rate of Australian Merino sheep with increasing worm burden. These authors argued that animals in an environment with a high worm burden were not able to show their genetic potential for growth in the same way than animals in an environment with a low worm burden could [49]. In short, parasite burden can affect animal performance with consequences for the heritability estimates.

The pairwise SNP correlations between BW at different ME and the genetic correlations between intercept and HR, indicate the presence of a genotype × parasite burden interaction for BW, which means that parasite burden might impact the EBV for BW, with consequences for selective breeding. It is important to consider parasite loads in selective breeding programs for BW and growth on pastured systems, especially in tropical areas. Varying levels of natural infestation, and different strategies for parasite control will impact animal performance and therefore affect genetic predictions as well. Tus, it is relevant to develop selection strategies that consider multiple breeding objectives, including phenotypes that might be indicators of animal health or parasite load, like the use of ImmuneDEX (IDEX) as selection criteria [50]. IDEX is an index that combines animal's ability to mount a cell-mediated immune response (Cell-IR) and an antibody-mediated immune response and can help in the identification of immune competent animals.

The unfavorable correlations between BW and HR to TICK were stronger for younger (ME.331) than older animals (ME.550). Tis result might be partially explained by the effect of age on immune response mechanisms [51–53]. Moreover, the association between animal size, skin surface and vasculature density might influence these unfavorable correlations [34]. Complementary studies are necessary to investigate the genetic mechanisms that underlie HR to different parasites at different growth stages. Considering the varying impact of HR at different ages, selection programs that measure BW at different ages, can develop a selection index to target multiple traits with a balancing approach, which might do a better job than targeting only BW at a young age. On the opposite side, selection for HR to GIN and HR to EIM will either benefit to or have no impact on BW, in general. These findings need to be further studied and validated on larger populations, and with more extreme parasite loads.

2.5.3. Candidate genes and pathways associated with host resilience

The methods, which were used here to search for functional candidate genes only in the QTL regions, filtered out the search for genes associated with HR to TICK and HR to GIN. In spite of the absence of significant QTL, some SNPs were significantly associated with these traits. For research purposes and with the main objective of avoiding the discussion of spurious associations from the GWAS, we believe that adding the QTL definition based on the presence of multiple significant and suggestive SNPs at a given region, is a good quality control to select for true associations. However, we do acknowledge that the population with available phenotypes was small, and that regions with significant SNPs associated with HR to TICK and HR to GIN might be within important QTL that were not identified because of the limitations due to the size of our database. Thus, future studies might bring other interesting insights on HR.

We identified several genes in the chemokine pathways, such as CXCL9, CXCL10, and CXCL11, which were related to HR to EIM. The transcripts of these genes are proinflammatory chemokines that are released from the intestinal epithelium [54]. The levels of both CXCL9 and CLCX11 transcripts were found to be increased in the gut tissue of susceptible mice that were artificially infected with *Trichuris muris*, but the up-regulation of these genes has not been verified in artificially-infected resistant mice [55]. Furthermore, in vivo neutralization of the CXCL10 gene resulted in a significant reduction in worm burden and increased rate of epithelial cell turnover in infected susceptible mice [56]. Clife et al. [56] demonstrated that CXCL10 had no effect on the TH1 immune response of susceptible animals, indicating that epithelial cell turnover alone can mediate worm expulsion. The CXCL9 gene plays an important role in antimicrobial defense by protecting the gut of artificially infected mice from the invasion by the bacteria *Citrobacter rodentium* and restoring the damaged tissue. These studies in mice suggest a possible mechanism underpinning the association of CXCL9, CXCL10, and CXCL11 with HR. The immune responses mediated by chemokines is probably an important mechanism for HR to all intestine parasites, including

protozoans. However, further studies in cattle are necessary to confirm the role that these genes might play in HR.

The modulators of antibody-mediated immune response, i.e. the IRS2, LIG4, and TNFSF13B genes were associated to HR to EIM in our study. These genes were also associated with human susceptibility to the nematode Ascaris lumbricoides, an endemic disease in tropical areas [57]. The TNFSF13B gene plays an important role in the class-switch recombination process and in the proliferation of B cells by rearranging its DNA sequence to switch their expression from one class of immunoglobulin, such as IgM, to an immunoglobulin heavy-chain constant region, which results in antibodies with different effector functions [58]. Moreover, the expression of TNFSF13B in the intestinal tissue of chickens that were orally infected with *Eimeria acervulina* increased after coccidiosis infection and led to a high antibody response [59]. Therefore, expression of HR to EIM can be associated with intestinal homeostasis maintenance and adaptive immune response. The genes that are significantly associated with HR to EIM were previously associated with nematode infections thus, it is possible that the defense mechanisms developed by animals exposed to GIN and EIM are partially similar. Further studies are required to validate these associations and the possible mechanisms that link the above discussed candidate genes with HR in cattle.

2.6. Conclusion

Selection under natural infestation and controlled parasite burden, via prophylactic parasite control, contributes to identify animals that are resilient to nematodes and Eimeria ssp. and that are expected to perform better under challenging environments (i.e. tropical regions). Chemokine pathways and intestinal epithelial cells are important for HR to gastrointestinal parasites and further studies focused on the expression of the candidate genes discovered in this study might help to better understand the HR mechanisms to different parasites.

2.7. Abbreviations

BW – body weight

CHR – chromosome

EBV – estimated breeding value

EIM – *Eimeria* spp.

EV-UFMG – Veterinary school of the Federal University of Minas Gerais

GIN – gastrointestinal nematodes

GRM – genomic relationship matrix

GWAS – genome-wide association study

HPD90 – high posterior density interval with 90% of samples

HR – host resilience

LD – linkage disequilibrium

ME – measurement event

QTL – quantitative trait locus

STM – single-trait linear random regression model

TICK – ticks

2.8. Declarations

2.8.1. Ethics approval and consent to participate

This study was realized with farm-owned animals with the farmer's approval. The Ethics and Animal Experimentation Committee of the Universidade Federal de Minas Gerais approved the experiment and data collection (Protocol 255/2010).

2.8.2. Consent for publication

Not applicable

2.8.3. Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

2.8.4. Competing interests

Author Daniel Resende Gonçalves was employed by the company Mundo Novo farm. The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

2.8.5. Funding

The genotyping process was funded by FAPEMIG (APQ-01609-16 Mapeamento de genes relacionados ao crescimento de bovinos de corte sob diferentes cargas parasitárias; APQ-00759-18 Avaliação genômica multirracial para características de interesse econômico em bovinos de corte), and CNPQ (461596/2014-8 Genes candidatos e vias biológicas associados com características de resistência a parasitos em bovinos Nelore).

2.8.6. Authors' Contributions

GG worked on planning the project of study, performed the analysis and wrote the paper. VR helped with data collection and with writing the paper. MF co-supervised the data analysis and helped writing this paper. FR co-supervised the data analysis and helped writing this paper. AG contributed to the statistical analysis and discussion of results. LP contributed to the statistical analysis and discussion of results. MM helped with data collection and with writing the paper. DG helped with data collection. MVS participated in the research funding and helped with writing the paper. FT participated in the planning of the project, research founding, co-supervised the data analysis, and helped writing this paper. All authors read and approved the manuscript submission.

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2.10. Supplementary Material

2.10.1. Additional file 1:

Table S1. Number of repeated measurements per animal.

Number of measurements	Number of animals
1	30
2	74
3	232
4	447
5	929

Table S2. Keywords used to construct the trained list of genes for body weight (BW) and host tolerance to ticks (HT.TICK), gastrointestinal nematodes (HT.GIN) and *Eimeria* spp. (HT.EIM).

Trait	GUILDify Keywords
BW	Body weight, Growth, Obesity, Protein, Muscle, Fat, Growth factors, Height
HT.TICK	Immunity, Immune response, Inflammation, Ectoparasite, Cytokines, Tick, Infection, Tolerance
HT.GIN	Immunity, Immune response, Inflammation, Endoparasite, Cytokines, Nematodes, Infection, Tolerance
HT.EIM	Immunity, Immune Response, Inflammation, Endoparasite, Cytokines, Eimeria, Infection, Tolerance

Table S3. Summary statistics of genes submits to candidate genes prioritization analysis for host tolerance to *Eimeria spp*.

Gene symbol	**Gene name**	**Average score**	**Overall *P-value***
CXCL9	C-X-C motif chemokine ligand 9	1.000	0.000
CXCL10	C-X-C motif chemokine ligand 10	1.000	0.000
CXCL11	C-X-C motif chemokine ligand 11	1.000	0.000
TNFSF13B	TNF superfamily member 13b [	0.771	0.000
SCARB2	scavenger receptor class B member 2	0.718	0.001
IRS2	insulin receptor substrate 2	0.639	0.001
SPINK5	serine peptidase inhibitor Kazal type 5	0.727	0.001
SPINK1	serine peptidase inhibitor Kazal type 1	0.710	0.002
HTR5A	5-hydroxytryptamine receptor 5A	0.607	0.005
COL4A1	collagen type IV alpha 1 chain	0.547	0.005
LIG4	DNA ligase 4	0.598	0.006
DPP6	dipeptidyl peptidase like 6	0.618	0.007
PTPRT	protein tyrosine phosphatase receptor type T	0.563	0.015
DPYSL3	dihydropyrimidinase like 3	0.504	0.020
NAAA	N-acylethanolamine acid amidase	0.544	0.034
STBD1	starch binding domain 1	0.360	0.042
NUP54	nucleoporin 54	0.391	0.064
SPINK6	serine peptidase inhibitor Kazal type 6	0.389	0.084
PAXIP1	PAX interacting protein 1	0.378	0.099
ART3	ADP-ribosyltransferase 3	0.277	0.144
PPEF2	protein phosphatase with EF-hand domain 2	0.383	0.145
SCGB3A2	secretoglobin family 3A member 2	0.348	0.191
MYO16	myosin XVI	0.274	0.222
STK32A	serine/threonine kinase 32A	0.270	0.224
SHROOM3	shroom family member 3	0.285	0.313
JAKMIP2	janus kinase and microtubule interacting protein 2	0.217	0.324
SDAD1	SDA1 domain containing 1	0.238	0.364
SOWAHB	sosondowah ankyrin repeat domain family member B	0.171	0.412
ABHD13	abhydrolase domain containing 13	0.110	0.500
CCDC158	coiled-coil domain containing 158	0.000	0.763

2.10.2. Additional file 2:

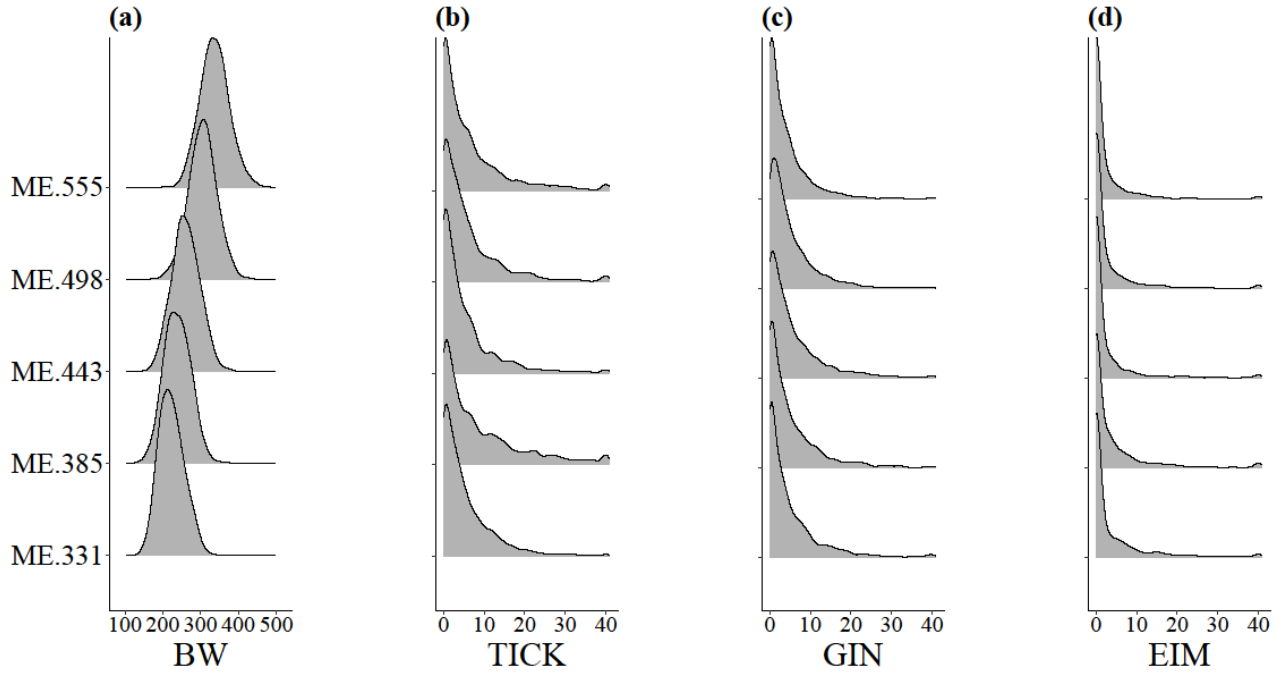

Figure S1. Distributions of body weight information (BW-**a**), ticks (TICK-**b**), gastrointestinal nematodes eggs (GIN-**c**), and *Eimeria* spp. oocysts (EIM-**d**) counts at each measurement event (ME). 331, 385, 443, 498, and 555 represent the mean ages of the animals at each ME, respectively.

2.10.3. Additional file 3:

Additional information on the methods used to process the statistical analysis

We used single trait linear random regression models (STM) to estimate the genetic parameters for host resilience to different parasites and the genetic parameters for body weight across a parasite burden trajectory. The model was previously described in the Methods section of the main text, and the analysis was accomplished through Bayesian methods by using Gibbs sampler.

For the STM, the prior assumptions and distributions were:

y was assumed as $y|b, a, G0, R1 \sim N(Xb + Za, IR1)$, where: $G0$ is the covariance matrix for the genetic additive effects, which were previously described in the Methods

section; R1 is the matrix of the residual effects, from which was considered homogeneity of variance so that $R1 = \left[\sigma^2_{e_1}\right]$. *X* and *Z* are the incidence matrices for the systematic (*b*) and genetic additive effects (*a*), respectively; *N* is the normal distribution; and *I* is an identity matrix with order equal to the number of observations.

The prior distribution for the effects were: $b \sim constant$; $[a_{0_{(i)}} \quad a_{1_{(i)}}]^t|A, G0 \sim N([0 \quad 0]^t, A \otimes G0)$; and $e|I, R1 \sim N(0, R1)$. Inverted Wishart distribution was assumed for the covariance matrix G0 (G0~IW(Σ^2_a, n_a) and Scaled inverse chi-squared distribution was assumed for σ^2_e ($\sigma^2_e \sim \chi^{-2}(v_e, S^2_e)$), where A is the relationship matrix, Σ^2_a and n_a are the hyperparameters for the Inverted Wishart distribution and v_e and S^2_e, are the hyperparameters for the scaled inverse chi-squared distribution. Non-informative priors were used. Information about a posteriori complete conditional distributions are also provided in Sorensen and Gianola [60].

Samples of the complete conditional distributions were obtained through the Gibbs sampler using the software GIBBS3F90 [61], with a chain of 1100000 iterations, discard of the 100000 first and sampling each 100 cycles. All the analyses were processed in sagarana HPC cluster, CEPAD-ICB-UFMG. The chain length was defined according to the method of Raftery and Lewis [62] in preliminary analysis, which is available in the BOA package [63] of the software R [26]. The convergence of the chains for each parameter of the model was evaluated by the criteria of Geweke [64] and Heidelberger and Welch [65], which are available in the same software and by visual inspection of the sampled values. For each parameter of the models the posterior means and high posterior density intervals with 90% of samples (HPD90) were calculated. The HPD90 was considered as a measure of uncertainty of the parameter estimate.

2.10.4. Additional file 4:

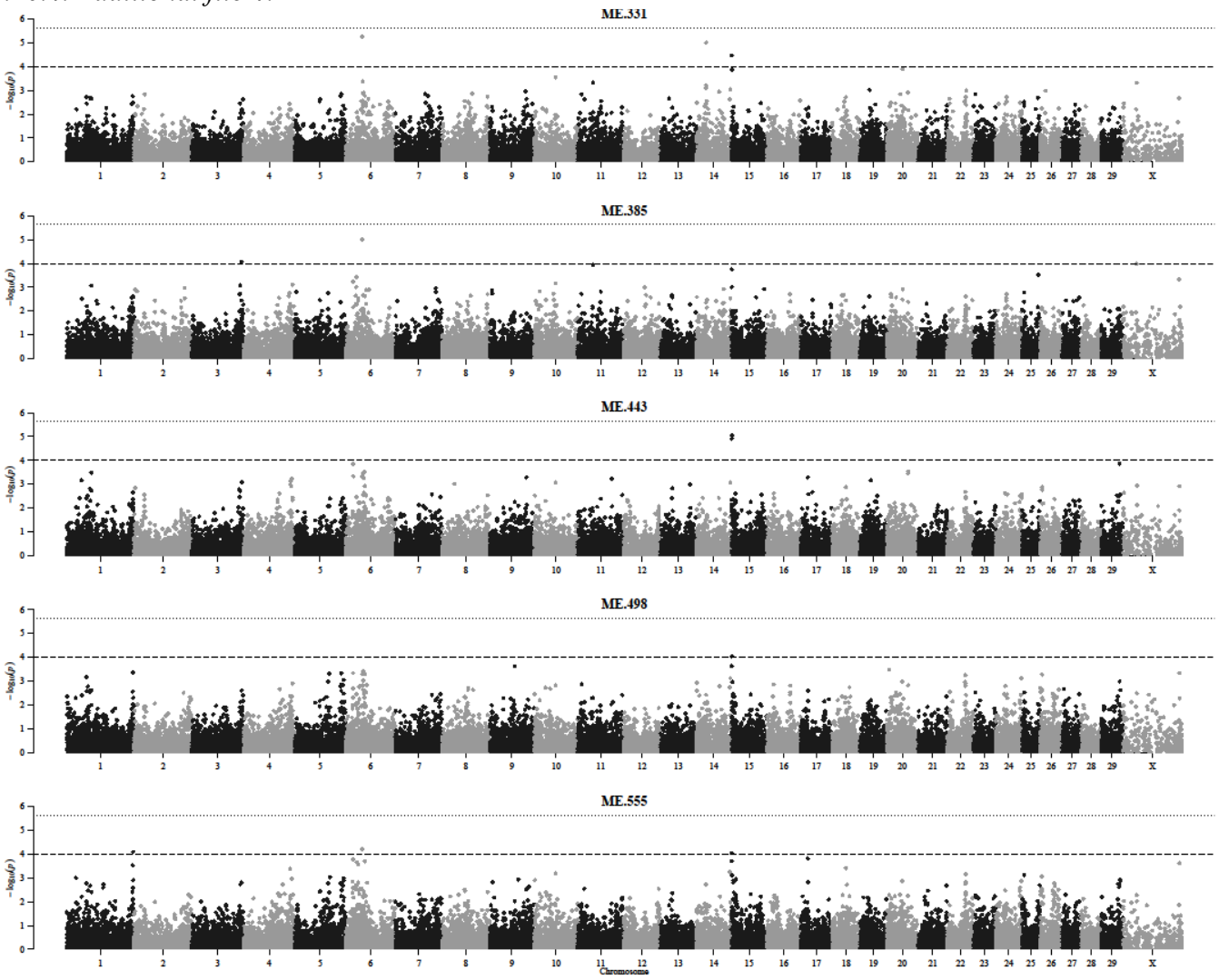

Figure S2. Manhattan plots for the genome-wide association studies for host tolerance to ticks evaluated at different measurement events (ME). 331, 385, 443, 498, and 555 are the mean ages (in days) of the animals at each ME. The dotted line (y = 5.64) indicates the threshold for statistical significance. The dashed line (y = 4.00) indicates the threshold for suggestive evidence of association.

2.10.5. Additional file 5:

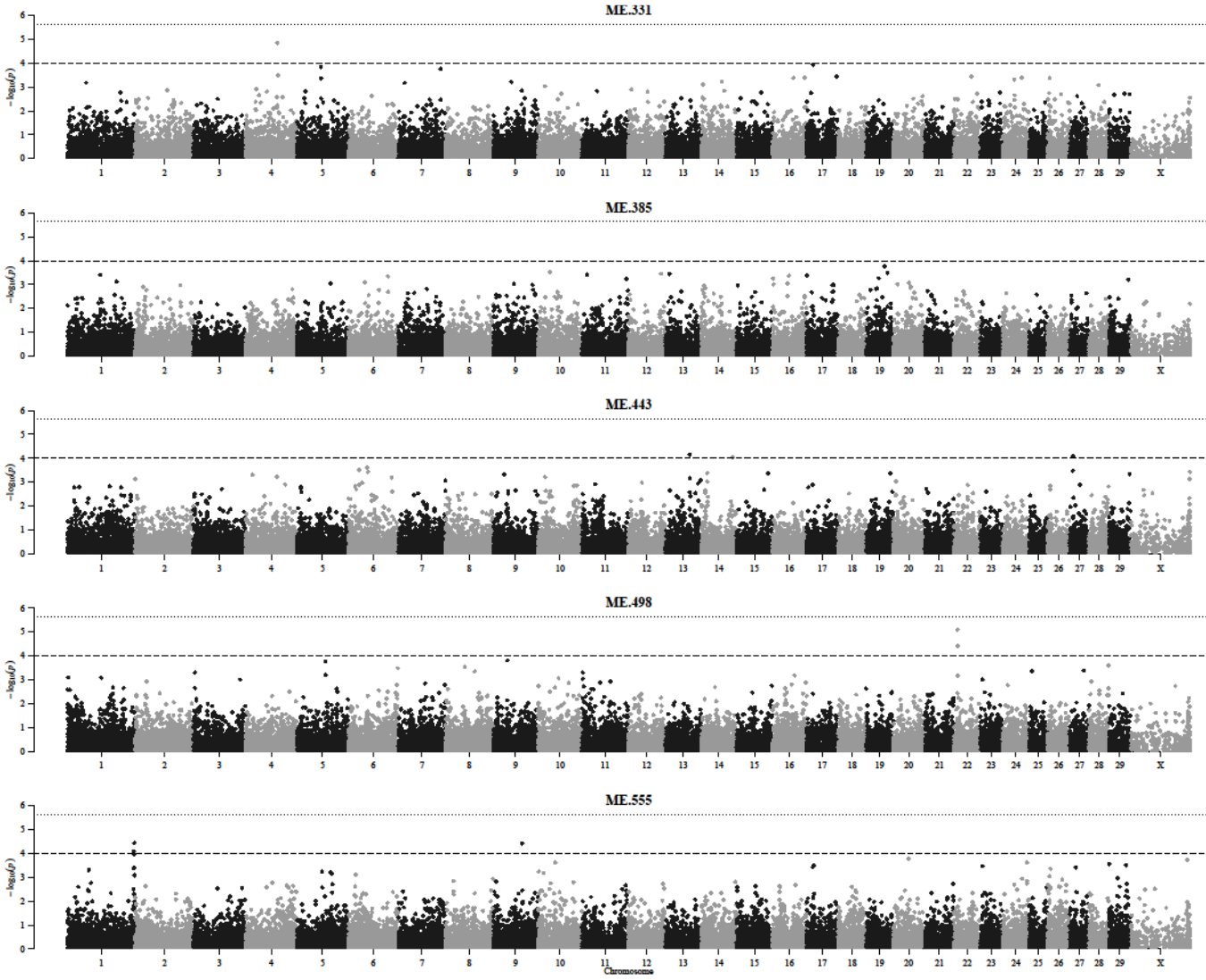

Figure S5 Manhattan plots for the genome-wide association studies for host tolerance to gastrointestinal nematodes evaluated at different measurement events (ME). 331, 385, 443, 498, and 555 are the mean ages (in days) that the animals had at the moment of evaluation. The dotted line (y=5.64) indicates the threshold for statistical significance. The dashed line (y=4.00) indicates the threshold for suggestive evidence of association.

2.10.6. Additional file 6:

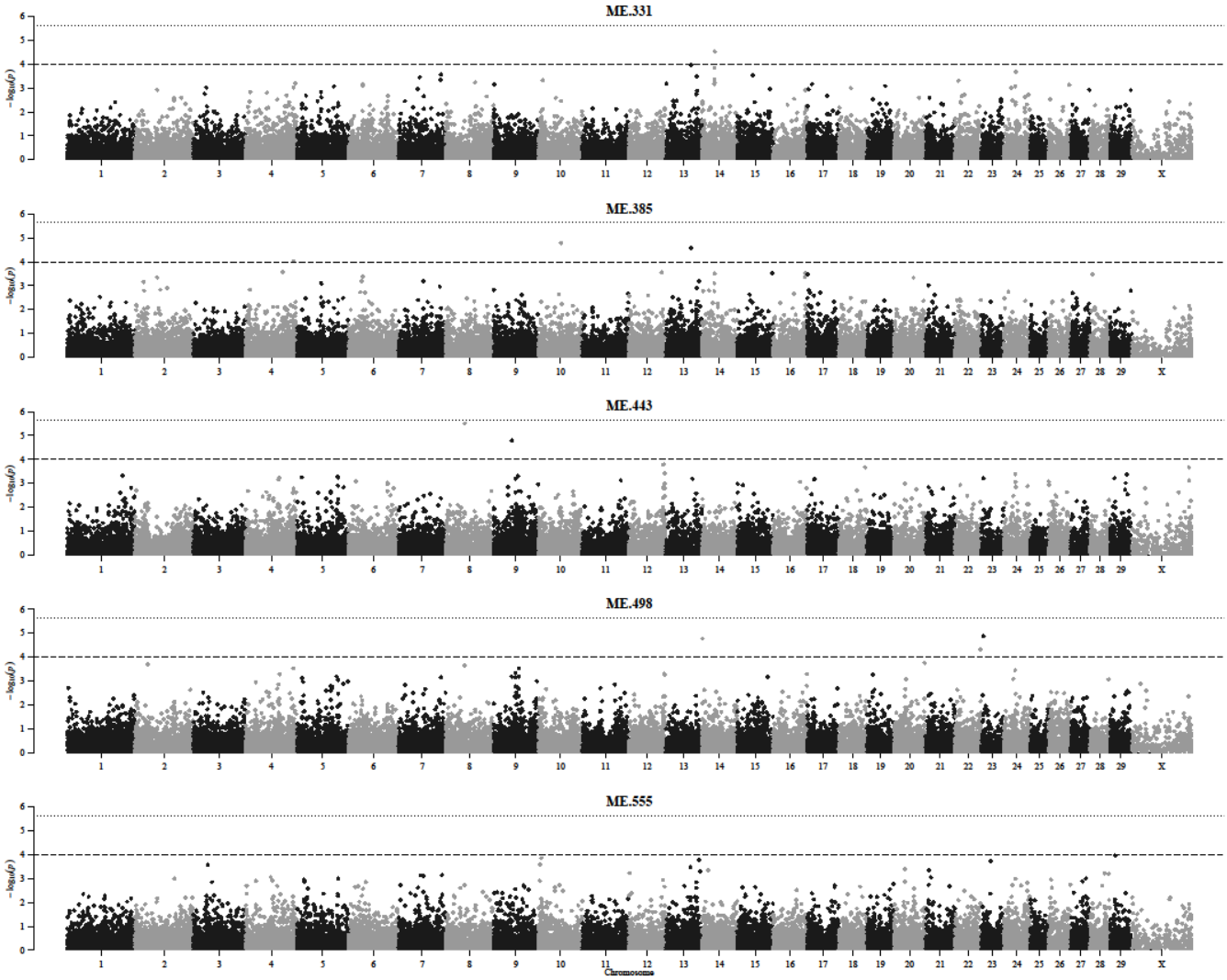

Figure S3.6 Manhattan plots for the genome-wide association studies for host tolerance to *Eimeria* spp. evaluated at different measurement events (ME). 331, 385, 443, 498, and 555 are the mean ages (in days) that the animals had at the moment of evaluation. The dotted line (y=5.64) indicates the threshold for statistical significance. The dashed line (y=4.00) indicates the threshold for suggestive evidence of association.

CAPÍTULO 3 – EVALUATION OF TICK RESISTANCE IN TROPICAL ADAPTED CATTLE FROM DIFFERENT GENETIC GROUPS

3.1. Introduction

Parasite burdens are known to cause significative losses in cattle productivity, influencing on the herd profitability. In tropical and subtropical regions, the detrimental impact of parasitism is accented since in these regions the environmental conditions are more adequate for the maintenance of the parasite lifecycle. From the different types of parasitosis that affect cattle population, ticks' infestations are responsible for the biggest economic impacts on cattle production, and around 80% of the world´s cattle are at risk of tick and tick-borne diseases [1].

Grisi et al. [2] estimated that around 14 million dollars were annually lost because of parasitosis in Brazilian cattle herds, being 3.2 million due to tick burden. In the past, the global loss caused by tick and tick-borne diseases to the cattle industry was estimated in about 18 billion dollars per year [3], and in Australia the annual costs of ticks for the northern beef industry was estimated at around 200 million dollars [4].

Besides of the use of chemical products, the selection for resistance have been suggested as an important tool to reduce the negative impacts of ticks and tick-borne diseases on cattle production [5–7]. Resistance can be defined as the ability of a host animal to limit its within-host pathogen load due to its capability to control or influence the parasite lifecycle [8–10].

Measurements which indicate level of parasite burden are often considered to be indicators of resistance [9]. For ticks, the gold-standard phenotype to evaluate host resistance is the number of engorging ticks on one or both sides of each animal following artificial or field infestation [1,11]. Genetic breeding values estimated for this phenotype have been used to select for more resistant animals and genomic technologies have been used to understand the biological mechanisms by which animals respond to tick infestations [6,12–14].

The genetic parameters estimated for tick counts (usually log-transformed tick counts - $TICK_{log}$) vary a lot between breeds. Some recent heritability estimates are from 0.11 to 0.25 for Nellore [6,15], from 0.10 to 0.40 for Hereford and Brahman [13], and 0.37 for a Bos taurus population – multiple breeds [14]. There is evidence that some of these differences can be due to differences in resistance of Bos taurus x Bos indicus subspecies [16,17]. Furthermore, we believe that differences on the genetic parameters might be a consequence of different mechanisms acting in the expression of a same trait, in different breeds [18,19].

In this regard, gene set enrichment analysis and candidate gene prioritization analysis are useful tools to study different mechanisms that might be associated with different phenotypes, or with a same phenotype evaluated in different breeds. These tools use gene expression information to identify groups of genes that share common biological function (gene set enrichment) [20] and that are prioritized, that means, that are similar to the enriched functions (candidate gene prioritization) [21].

The aim of the present study was to estimate genetic parameters for tick counts in two different beef cattle populations of Nellore and Tropical Composite breeds. Also, we aimed to verify the impact of mixed breeds composition on the tick burden, regarding

the different origin of alleles (Taurine x Indicine subspecies). Differences on genetic mechanisms by which animals of the different breeds respond to tick burden were also described.

3.2. Methods

3.2.1. Data collection and edition

3.2.1.1. Nellore cattle

Phenotypes and genotypes from Nellore animals raised in a commercial farm named Mundo Novo, located in Uberaba, Minas Gerais state, Brazil (19° 24'33 "S and 48° 06'34" W, altitude of 840 meters, Monsoon-influenced humid subtropical climate or Cwa weather according to Köppen scale), were used in the presents study. Animals were born between 2011 and 2016. The experimental data was collected during performance tests at pasture. The Ethics and Animal Experimentation Committee of the Universidade Federal de Minas Gerais approved the experiment and data collection (Protocol 255/2010).

The farm description, where the performance tests were realized, can be found at Passafaro et al. [5]. The performance test scheme is described at Gouveia et al. [11] Samples collected at the first intermediate and final measurement events were considered as yearling (P.365) and over yearling (P.550) information, respectively.

In each event animals were weighted and engorged female ticks (length size > 4.5 mm) counts were performed on the right side of each animal. The length size of the engorged female ticks were defined according to the technique proposed by Wharton and Utech

[12]. The tick counts were not multiplied by any constant (in some studies they are multiplied by two as a estimative of the counts observed in the entire animal [23]).

Animals were subjected to natural parasite infestation, and approximately 65% of the bulls were prophylactically treated at the beginning of the performance tests (day 1 of adaptation period) with Ivermectin 4% (1ml of Ivermectin per 50Kg of live BW - Master LP, Ouro Fino Saúde Animal, Cravinhos, SP). The treated bulls were randomly chosen by contemporary group, in such a way that we had some entire groups treated or not. The contemporary groups were defined as the group of animals that were raised together in the same paddock. Information of animals belonging to contemporary groups with less than five animals were removed. Only animals with both phenotypic and genotypic information were considered.

Blood samples were collected from the coccygeal vein with sterilized syringes into vacuum tubes of 3.5 ml containing 9NC Coagulation Sodium Citrate 3.2%. They were frozen and transferred to the Laboratory of Genetics at EV-UFMG, where they were stored in freezers at -20ºC. 1230 blood samples were selected for genotyping with a low-density DNA array: the Z-chip v2 (Neogen, Lincoln, Nebraska, EUA, which genotypes – 27533 SNPs). Details about samples selected for genotyping can be found at Gouveia et al. [11].

Quality control of SNPs and samples were made using the SNPStats R-package [24]. Only SNPs located at autosomal and X chromosome, with call rate > 0.95 and minor allele frequency > 0.05 were considered in the panel. Duplicated samples (correlation between samples > 0.95) were removed. Only samples with call rate > 0.95 were considered in the present study. After the quality control procedure, the low-density SNP panel was formed by 21667 SNPs and 1075 samples.

The previously referred SNP panel (named here as target panel) was imputed to a high-density panel (700k SNPs). From the 21667 SNPs that were kept at the low-density panel after quality control, 20282 were also present at the high-density panel. The reference population for the imputation was formed by 2354 Nellore animals (231 being from Mundo Novo herd) genotyped with the Illumina HD array (Neogen, Lincoln, Nebraska, EUA, with 777962 SNPs). The same previous quality control restrictions were applied for the reference panel, resulting in a final panel with 2049 animals and 465631 SNPs.

The imputation was done in each chromosome separately. First, the phasing was carried out at Eagle V2.4.1 [25] for both target and reference panel separately. Default parameters were used to do the phasing, including the imputation of missing genotypes within each panel. The phased haplotypes that Eagle outputs contain best-guess imputed genotypes. After phasing, the imputation was realized using Minimac4 [26]. For this, the phased files for the X chromosome were separated into the pseudo autosomal and the non-pseudo autosomal region using VCFTools software [27], and the reference panel was converted into M3VCF format using Minimac3 (see https://genome.sph.umich.edu/wiki/Minimac4). Information regarding both the target and reference panels used in the imputation process can be verified at Table 3.1.

Table 3.1. Description[1] of both target and reference panels used for imputation of Nellore genotypes

Chromosome[2]	$SNP_{reference}$	SNP_{target}	%SNPs to be imputed
1	28014	1264	95.49
2	24172	1076	95.55
3	21915	1037	95.27
4	20107	943	95.31
5	19415	929	95.22
6	23493	976	95.85
7	20049	879	95.62
8	21683	912	95.79
9	20053	899	95.52
10	17046	803	95.29
11	18572	830	95.53
12	14971	647	95.68
13	13845	642	95.36
14	16741	669	96.00
15	14394	692	95.19
16	14512	676	95.34
17	13663	595	95.65
18	11443	509	95.47
19	10335	523	94.94
20	12459	623	95.00
21	12346	570	95.38
22	10333	473	95.42
23	9415	480	94.90
24	11091	509	95.41
25	7135	339	95.25
26	9272	425	95.42
27	8020	343	95.72
28	7818	344	95.60
29	7921	371	95.32
X*	22916	225	99.02
PAR*	2492	79	96.83

[1]$SNP_{reference}$ = number of SNPs in the reference panel; SNP_{target} = number of SNPs in the target panel; *X = non-pseudo autosomal region of X chromosome; PAR = pseudo autosomal region of X chromosome.

Minimac4 calculates squared correlation between imputed genotypes and true, unobserved genotypes (Rsq – see https://genome.sph.umich.edu/wiki/Minimac3_Info_File) for each SNP. Only SNPs with Rsq > 0.4 were considered (3370 SNPs were removed). The average Rsq for the realized imputation was 0.98.

The imputed files of each chromosome were merged, and the imputed panel was formed by 1462 animals of Mundo Novo farm with information of 462261 SNPs. After exclusion of SNPs with minor allele frequency < 0.05, the final panel was formed by 1462 animals with information of 421361 SNPs. From these, 1161 animals had phenotypic information in at least one of the studied periods (P.365 or P.550). The summary statistics for the phenotypic data of Nellore cattle at P.365 and P.550 are described at Table 3.2.

Table 3.2. Summary statistics[1] for tick counts (TICK), body weight (BW), and age of Nellore cattle evaluated at yearling (P365) and over yearling (P.550)

Trait	N	Mean	sd	Median	Min	Max
			P.365			
TICK	976	9.29	11.76	5.00	0.00	131.00
BW	976	244.05	33.05	243.50	135.00	377.00
Age	976	387.11	22.93	390.00	339.00	428.00
			P.550			
TICK	1126	7.40	9.17	4.00	0.00	72.00
BW	1126	343.37	36.65	343.50	214.00	253.00
Age	1126	555.25	23.40	558.00	502.00	595.00

[1] N = number of observations; sd = standard deviation; Min = minimum; Max = maximum

2.2.1.2. Tropical composite cattle

The Tropical Composite data used for the present study was collected previously and is managed by researchers of the Commonwealth Scientific and Industrial Research Organization. Animal Care and Use Committee approval was not necessary since no new animals were evaluated for the realization of this study.

The Tropical Composite is a population formed by crosses of *Bos indicus* and *Bos taurus* breeds (50% *Bos indicus*, *African Sanga* or other tropically adapted *Bos taurus*; 50% non-tropically adapted *Bos taurus*) [28]. The animals were raised in northern

Australia, under tropical and subtropical conditions. Both the cattle management, and population description can be found at Pragaya et al. [29] and Barwick et al. [28]. Animals that had information about tick score instead of tick counts were discarded from the data set.

The data set was from 747 animals with up to 3 records by animal, totalizing 1578 records. Age restrictions were applied to define which records would be considered as yearling and over yearling information. For this, we defined two age intervals: 365 ± 90 days (yearling data set - P.365) and 550 days ± 90 days (over yearling data set - P.550) to select the records. If inside these intervals more than one record was collected for one animal, we considered as P.365 or P.550 the record collected closest to the animal's 365 or 550 days old, respectively.

Tick counts were realized according to the Wharton and Utech [12] technique. The evaluated count represents the number of ticks observed on one side of the animals, without multiplying by any constant. The proportion on *Bos indicus* of each animal was estimated through admixture analysis [30]. Contemporary groups were formed by the combination of the date in which the measurement was made and the animal sex. Contemporary groups with less than five animals were removed from the data set. All the evaluated animals had both phenotypic and genotypic information.

The genotypic panel was formed by 722208 SNPs. Duplicated samples (correlations between samples > 0.95) were removed from the data set. Only SNPs with minor allele frequency > 0.05 were kept in the panel after quality control. The final panel was formed by 668678 SNPs of 711 animals. Summary statistics for the P.365 and P.550 data sets are presented in Table 3.3.

Table 3.3. Summary statistics for tick counts (TICK), body weight (BW), age, and percentual of Indicus composition (%Zebu) of Tropical Composite cattle in the complete, yearling (P.365), and over yearling (P.550) data sets

Trait	N	Mean	sd	Median	Min	Max
			P.365			
TICK	416	29.41	35.07	16.00	0.00	221.00
BW	416	237.85	42.76	238.00	126.50	358.00
Age	416	394.32	37.23	389.50	295.00	455.00
%Zebu	416	0.25	0.08	0.25	0.05	0.60
			P.550			
TICK	588	23.56	26.31	16.00	0.00	195.00
BW	588	308.25	64.55	301.00	167.00	562.00
Age	588	495.11	37.39	483.00	460.00	640.00
%Zebu	588	0.25	0.08	0.25	0.05	0.58

[1] N = number of observations; sd = standard deviation; Min = minimum; Max = maximum

3.2.2. Statistical models

3.2.2.1. Data distribution

Tick counts were used as indicator of host resistance to ticks. For both datasets (P.365 and P.550) and for both breeds (Nellore and Tropical Composite), tick distributions did not follow normal distribution (Figure 3.1), which is a presupposition of most of linear models traditionally used to estimate genetic parameters. For this reason, logarithm transformations were used for all the evaluated tick counts [6,15], in which:

$$TICK_{log} = log_{10}(TICK + 1.001),$$

in which $TICK_{log}$ represent the tick counts after logarithmic transformation, and TICK represent the observed number of TICK on the right side of the evaluated animal. The constant 1.001 was included since some counts were equal to zero [6,31].

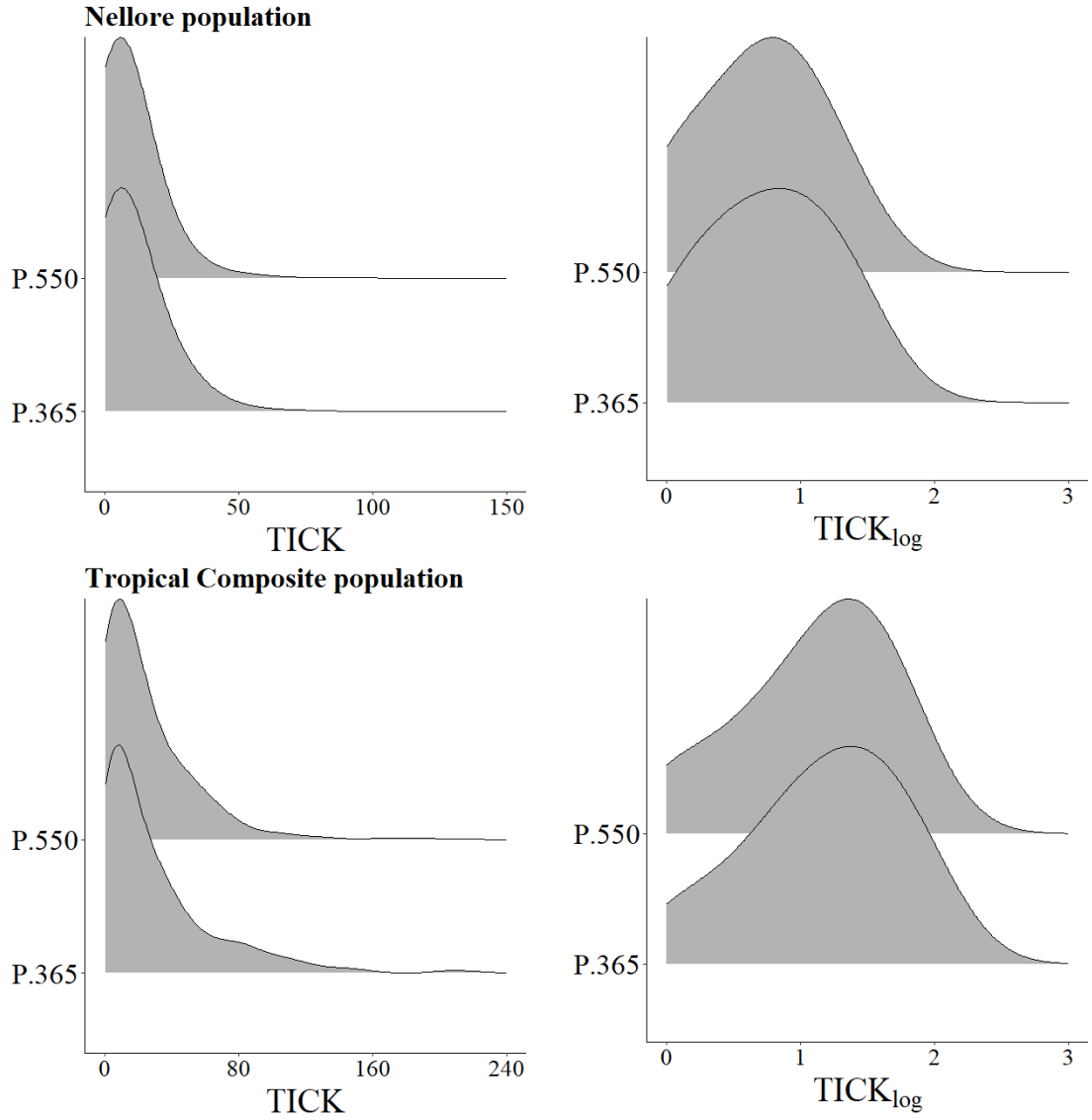

Figure 3.1. Distribution of tick count (TICK) and log-transformed tick counts ($TICK_{log}$) of yearling (P.365) and over yearling (P.550) beef cattle of Nellore and Tropical Composite breeds.

3.2.2.2. Fixed effects

The influence of age, body weight, contemporary groups, and percentual of *Bos Indicus* in the genetic composition of the animals (%Zebu, only for Tropical Composite data

set) on the $TICK_{log}$ was evaluated using the *lm* function of R software [32]. The quantitative effects were tested individually, in such way that:

$$TICK_{log_{ijk}} = a_i + b_j x + e_{ijk},$$

where $TICK_{log_{ijk}}$ represents the tick counts after logarithmic transformation, a is the intercept that estimates the mean solution *i* for the trait disregarding other effects, b is the angular coefficient *j* estimated for the fixed effect x, x represent the observed age, or body weight, or %Zebu that animals were in the evaluation, and e is the random residual estimated for each observation *j* of the model.

The contemporary group, that is a classificatory effect was tested as follow:

$$TICK_{log_{ijk}} = a_i + b_j GC + e_{ijk},$$

a is the intercept that estimates the mean solution for the first contemporary group, b estimates the mean difference estimated between each contemporary group CG (from the second to the last) and the first group (estimated by the intercept), and e is the random residual estimated for each observation of the model.

After defining all the effects that significantly influenced the traits, a final additive model was tested to verify if the effects remained significant when considered together at the genetic evaluations. The genetic evaluations were processed through single trait animal models.

The solutions and standard deviations estimated for fixed effects at both linear regressions and single-trait animal models were used to calculate 95% Confidence Intervals for the parameters. The intervals were used to infer about the significance of the effects and were calculated as:

$CI95\% = \bar{X}_{Sol} \pm 1.96xsd_{sol}$, where CI95% is the confidence interval for typical response [33], $\bar{X}_{sol}$ is the mean solution estimated for the effect, and sd_{sol} is the standard deviation estimated for the effect. The null hypothesis tested by CI95% was that the $\bar{X}_{sol} = 0$, when CI95% included the zero value, the null hypothesis was not rejected.

3.2.2.3. Variance Components

As it was previously informed, genetic evaluations were processed through single trait animal models. The variance components were estimated with these models. The matrix notation of the final model is:

$$y = X\beta + Z\alpha + e,$$

where y is a vector of log transformed tick counts measured in each age; X is the incidence matrix for the fixed effects (contemporary groups for Nellore data sets, and contemporary groups and genetic composition for Tropical Composite data sets); β is a vector of solutions for the fixed effects; Z is the incidence matrix of the genetic additive genetic random effects; α is the vector of solutions for the random effects, that contains the genomic estimated breeding values (GEBV) for $TICK_{log}$ of each animal; e is a vector of random residual associated to each observation. Resistance was evaluated separately for each age under study.

The following priori were used to process the model: Flat distributions for fixed effects β (β ~ constant); normal distributions for the genomic estimated breeding values α (α | GRM, σ_α^2 ~N(0, GRMσ_α^2)) and for the residuals associated to each observation (e | σ_e^2 ~ N(0, Iσ_e^2)); and scaled inverse chi-squared distributions for the variances of random

effects $\sigma_\alpha^2 \sim X^{-2}(v_a, S_a^2)$ and $\sigma_e^2 \sim X^{-2}(v_e, S_e^2)$. The GRM is the genomic relationship matrix estimated according to VanRaden et al. [34], I is an identity matrix with order equal to the number of observations, σ_α^2 is the additive genetic variance; σ_e^2. Is the residual variance, v_a, v_e, S_a^2, and S_e^2 are the hyperparameters for the scaled inverted chi-squared distributions. Only the markers positioned on the autosome chromosomes were considered for calculating the GRM since dosage compensation methods are not available at BLUPF90 family programs [35–37]. This way, 401177 and 639868 SNPs were used to estimate the GRM for Nellore and Tropical Composite populations, respectively. Information about a posteriori complete conditional distributions are also provided in Sorensen and Gianola [38]. The genetic evaluations were performed using GIBBS3F90 software [39]. Chains of 550000 iterations were considered, with discard of 50000 iterations and sampling each 50 iterations. The software uses a Bayesian method through Gibbs sampler.

For each sample of the posterior distribution of the parameters of the model, phenotypic variances (σ_p^2) were calculated as $\sigma_p^2 = \sigma_\alpha^2 + \sigma_e^2$, heritability ($h^2$) as $h^2 = \frac{\sigma_\alpha^2}{\sigma_p^2}$, and additive genetic variance coefficients (CV_α) as $CV_\alpha = \frac{\sqrt{\sigma_\alpha^2}}{\overline{TICK_{log}}}$, where $\overline{TICK_{log}}$ is the mean of log-transformed tick counts observed in each data set, in such a way that posterior distributions (and high posterior density intervals with 90% of samples) were also constructed for these parameters.

3.2.3. Genome wide association studies

3.2.3.1. SNP Solutions

Genome-wide association for $TICK_{log}$ were realized for each data set separately (P.365 and P.550 for Tropical Composite and Nellore cattle). The GEBV were predicted using BLUPF90 software [39] and both SNP solutions and p-values associated to them were calculated according to Aguilar et al. [40] using POSTGSF90 software [41].

The significance of SNP solution on the $TICK_{log}$ was defined using a Bonferroni correction for multiple tests, in which two thresholds were defined: one for identifying significant SNPs, that was defined as $\alpha_1 = \frac{0.05}{n}$, in which α_1 is the threshold for significance and n represent the number of SNPs considered in the markers panel; and the second for suggestive SNPs, that was defined as $\alpha_2 = \frac{1.00}{n}$, in which α_2 is the threshold for suggestive SNPs [42,43].

3.2.3.2. Genes associated with $TICK_{log}$ in different beef cattle populations

We defined windows of 0.5Mbp upstream and downstream the suggestive or significant SNPs [6,44] and looked for genes located inside these windows around suggestive SNPs using the ARS-UCD1.2 bovine genome assembly (available at ftp.ensembl.org/pub/release-103/gtf/bos_taurus/). The search for genes was made using the GALLO package [45] of R software [32]. The identified genes were considered as positional candidate genes for $TICK_{log}$.

Known genes shared between at least two data sets (P.365-Nellore x P.550-Nellore, or P.365-Tropical Composite x P.550-Tropical Composite or P.365-Nellore x P.550-Tropical Composite or P.365-Tropical Composite x P.550-Nellore) were selected for a

candidate gene prioritization analysis. Candidate gene prioritization analysis require a training gene list and a target gene list formed by the positional candidate genes. The training gene lists was constructed by the first one hundred genes that were associated to a group of keywords used to describe $TICK_{log}$. The training gene list was built on GUILDify v2.0 [46], using the keywords immunity, immune response, inflammation, ectoparasite, cytokines and tick [6]. These keywords were used to search for genes in the Biologic Interaction and Network Analysis (BIANA) knowledge database.

At GUILDify, genes associated with the user-provided keywords are used as seeds to generate protein interaction networks. The networks were generated for *Homo sapiens* since bovine was not an option. The NetScore prioritization algorithm from the GUILD package was used to generate the prioritization scores of the identified BIANA entries. The GUILDify default options were used at the NetScore algorithm (repetition = 3 and iteration = 2).

The target gene list was formed by genes shared between at least two data sets, as previously informed, that have orthologous genes in *Homo sapiens* (that was used as the reference specie), and that were not already considered in the training gene list. Both training and target gene lists were used for candidate gene prioritization analysis at ToppGene Suite [22].

We used ToppGene function, that perform the prioritization analysis in two-steps. First, an enrichment analysis was realized to identify functional information shared between the trained and the target lists. The following training parameters were used to look for the functional annotation: Gene ontology terms (molecular function, biological process, and cellular component), human and mouse phenotypes, pathway, PubMed, transcription factor binding site, coexpression, and disease.

For the enrichment analysis, a random sampling of the entire genome was made each 5000 genes, and for each training parameter, the number of genes we are interested in was compared with the number of genes in the random genome sampling, using a statistical meta-analysis. A Bonferroni correction for multiple tests was used to identify the significant pathways of each training parameter ($p<0.05$).

At the second step, ToppGene function calculates a similarity score for each gene in the training data set with each training parameter, as well as a p-value associated with this score [22]. These scores are based on fuzzy-based similarity measures for the categorical terms [47], and on Pearson correlations between the genes in the training and in the genes in annotation for each quantitative training parameter. In the case of a missing value (for instance, lack of one or more annotations for a test gene), the score is set to −1. Otherwise, it is a real value in [0, 1] [22]. Afterall, an overall similar score is calculated, as well as a p-value associated with the overall score. Genes with overall p-value < 0.05 were considered here as the prioritized genes. The genes in common associated with at least two data sets, and that were submitted to prioritization analysis, were evaluated in three different groups: genes shared between P.350 and P.550 of Nellore cattle only; genes shared between P.350 and P.550 of Tropical Composite cattle only; and genes shared between Nellore and Tropical Composite cattle (all possible combination of data sets).

3.3. Results

3.3.1. Statistical models

3.3.1.1. Fixed effects

The effect of contemporary groups was the only significant for $TICK_{log}$ of yearling and over yearling Nellore cattle, according to fixed linear regressions. Thirty-six and 40 contemporary groups were evaluated at P.365 and P.550, respectively. As contemporary group is a categorical variable, we considered that if at least one contemporary group presented significant effect ($p< 0.05$) at the fixed models, the effect was considered as significant. Furthermore, for each model (P.365 and P.550), the p-value associated with the F-statistic was $P < 0.001$, indicating that there is at least one orthogonal contrast statistically different from zero. The adjusted R-squared, that is a statistic that indicates the model fit, were 0.67 for the model adjusted for P.365, and 0.45 for the model adjusted for P.550.

For Tropical Composite cattle, the effects of contemporary group and breed composition were significant for $TICK_{log}$ at fixed linear regression models. A final additive model was adjusted considering both effects as covariables. For both data sets of Tropical Composite, the p-value for the F-statistic was $P<0.001$. The Adjusted R-squared of the models were 0.37 for P.365, and 0.36 for P.550.

At the fixed linear regression models, negative solutions (mean ± standard deviation) of -1.84 ± 0.30 and -1.39 ± 0.25 were observed for the effect of %Zebu on the $TICK_{log}$ for both yearling and over yearling evaluations, respectively. At the single-trait animal models, the estimated solutions for %Zebu were also negative (-2.29 ± 0.54 for P.365, and -1.76 ± 0.42 for P.550). The confidence intervals constructed to determine the significance of %Zebu on $TICK_{log}$ measured at the Tropical Composite population are presented at Table 3.4.

Table 3.4. Mean ($\bar{X}_{sol}$), standard deviations (sd_{sol}), and limits of confidence intervals with 95% of confidence (CI95%) of the %Zebu effect on the log-transformed tick counts of yearling (P.365) and over yearling (P.550) Tropical Composite cattle

Period	Linear regression models			Single-trait animal models		
	$\bar{X}_{sol}$	sd_{sol}	CI95%	$\bar{X}_{sol}$	sd_{sol}	CI95%
P.365	-1.84	0.30	-2.43; -1.25	-2.29	0.54	-3.35; -1.23
P.550	-1.39	0.25	-3.13; -0.90	-1.76	0.42	-2.58; -0.94

3.3.1.2. Variance components

The genetic parameters estimated for $TICK_{log}$ for all the studied breeds in both periods are present at Table 3.5. For each breed and between the different periods, there is no significant difference observed on the parameters between yearling and over yearling evaluations since the highest posterior density intervals with 90% of samples (HPD90) overlapped.

Table 3.5. Posterior means (and highest posterior density intervals with 90% of samples – HPD90) of the additive genetic (σ_a^2), residual (σ_e^2), and phenotypic (σ_p^2) variances, heritability (h^2), and coefficients of additive genetic variance (CV_a) for log-transformed tick counts ($TICK_{log}$) of Nellore and Tropical Composite cattle evaluated at yearling (P.365) and over yearling (P.550)

	σ_a^2	σ_e^2	σ_p^2	h^2	CV_a (%)
			Nellore*		
P.365	0.20	7.61	7.81	0.03	5.18
	(0.00; 0.46)	(7.00; 8.28)	(7.21; 8.39)	(0.00; 0.06)	(0.59; 9.38)
P.550	0.26	11.36	11.62	0.02	6.40
	(0.00; 0.60)	(10.47; 12.24)	(10.83; 12.47)	(0.00; 0.05)	(0.89; 11.46)
			Tropical Composite		
P.365	0.11	0.11	0.23	0.49	15.13
	(0.05; 0.17)	(0.07; 0.16)	(0.20; 0.25)	(0.26; 0.72)	(10.99; 19.57)
P.550	0.06	0.14	0.21	0.30	11.79
	(0.03; 0.10)	(0.11; 0.18)	(0.19; 0.23)	(0.15; 0.46)	(8.42; 15.17)

*trait X 10^2

Low heritabilities were estimated for $TICK_{log}$ in Nellore cattle, while moderate to high heritability were observed for the same trait in Tropical Composite cattle (Table 3.5). The same trend was observed for coefficients of additive genetic variance.

3.3.2. Genome wide association studies

The thresholds for considering SNPs as significantly or suggestively associated with $TICK_{log}$ in Nellore cattle were 1.25 x 10^{-7} and 2.49 x 10^{-6}, respectively. Six SNPs

located at chromosomes 1, 10, 23, 28, and 29, were significantly associated with $TICK_{log}$ at P.365 (Figure 3.2). At P.550 we did not find significant associations. For both periods, suggestive associations were found: 109 and 74 SNPs for P.365 and P.550, respectively. There is no overlap of significant or suggestive SNPs between P.365 and P.550. Details about the associated markers can be found at Table S1 for P.365 and Table S2 for P.550.

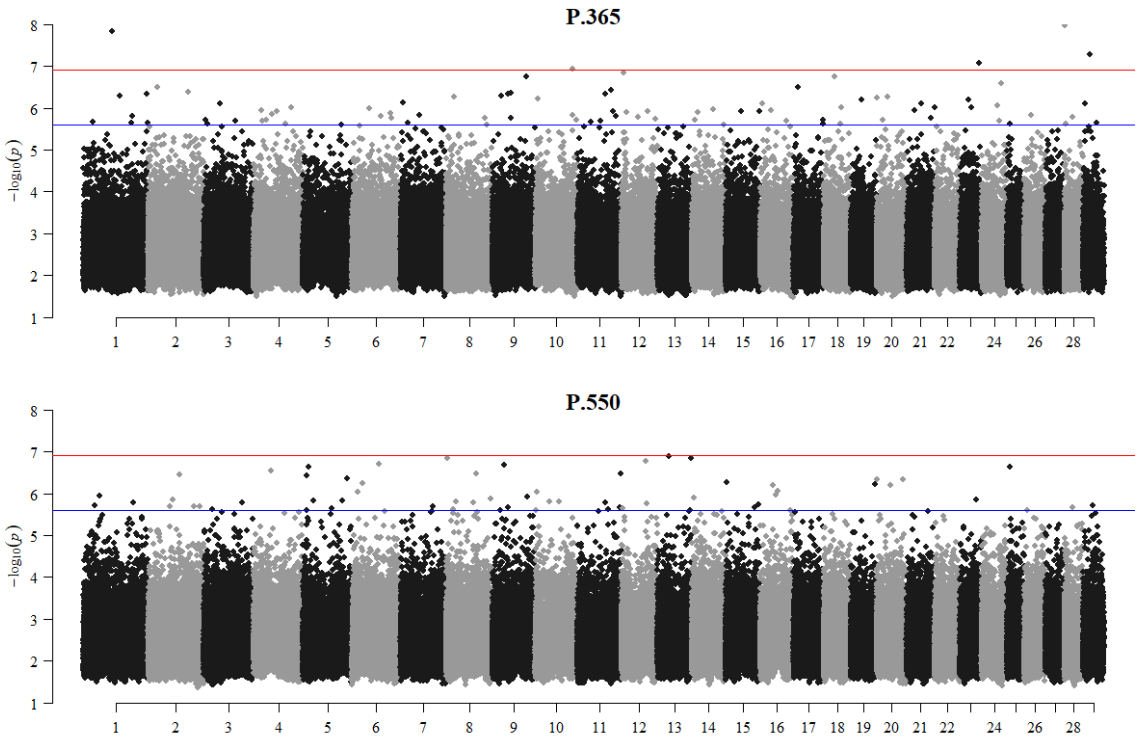

Figure 3.2. Manhattan plots for the genome-wide association studies of log-transformed tick counts of yearling (P.365) and over yearling (P.550) Nellore cattle. The blue and red lines represent the threshold for suggestive ($-\log_{10}(p)=5.60$) and significant associations ($-\log_{10}(p)=6.90$), respectively.

For the GWAS realised for Tropical Composite data, the threshold used to consider the SNP effect as significant for $TICK_{log}$ was 7.81×10^{-7}, and 1.56×10^{-6} for suggestive SNPs. For $TICK_{log}$ at P.365, we did not identified markers with significant effect

(Figure 3.3). Fifty SNPs with suggestive effect were identified for $TICK_{log}$ at P.550 (Figure 3.3, Table S3).

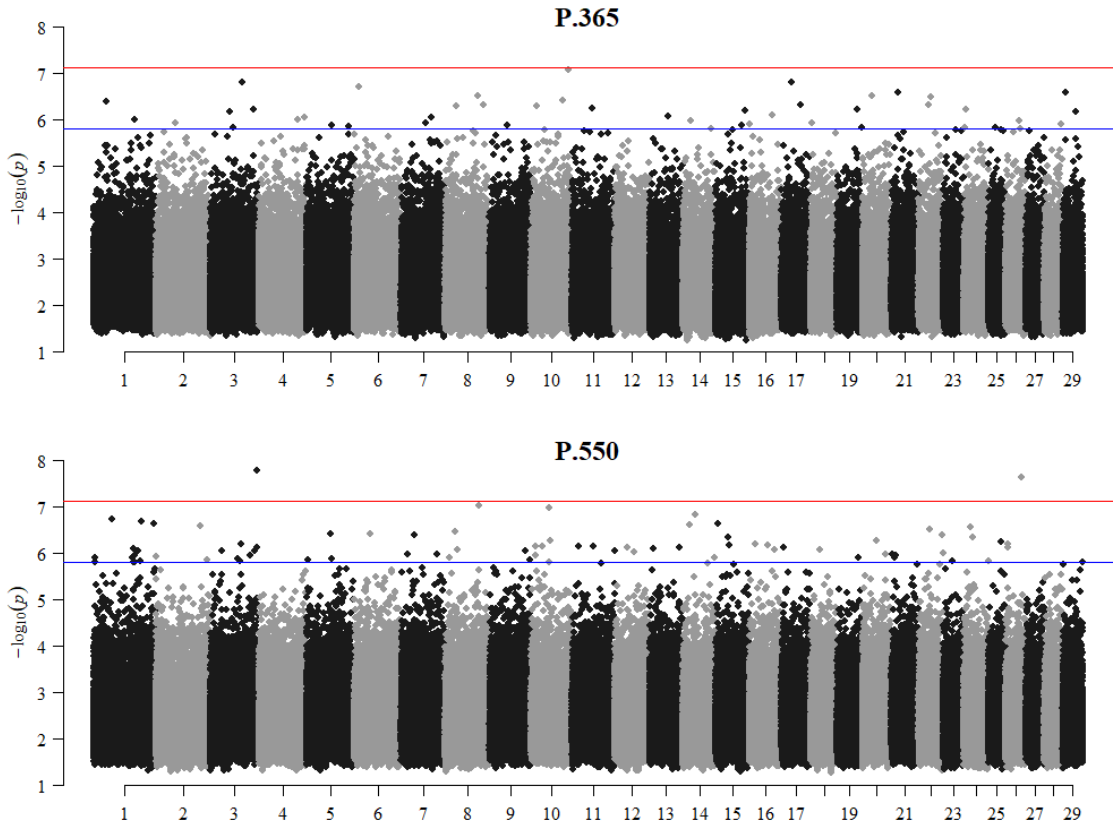

Figure 3.3. Manhattan plots for the genome-wide association studies of log-transformed tick counts of yearling (P.365) and over yearling (P.550) Tropical Composite cattle. The blue and red lines represent the threshold for suggestive ($-\log_{10}(p)=5.81$) and significant associations ($-\log_{10}(p)=7.11$), respectively.

For P.550 of Tropical Composite cattle, 3 SNPs were significantly associated with $TICK_{log}$: at the chromosome 3 and 26 (Figure 3.3, Table S4). There was not overlap between SNPs suggestively associated at P.365 with the significant or suggestive associations observed at P.550.

Information about genes associated to each suggestive or significant SNPs associated to $TICK_{log}$ of both Nellore and Tropical Composite cattle at P.365 and P.550 is presented at Table S5 for Nellore at P.365, Table S6 for Nellore at P.550, Table S7 for Tropical Composite at P.365, and Table S8 for Tropical Composite at P.550.

Forty-five genes (2.57% of all known genes associated with $TICK_{log}$) were associated with $TICK_{log}$ in at least two data sets (Figure 3.4). These gene IDs are presented at Table 3.6 and formed the target gene list for the candidate gene prioritization analysis.

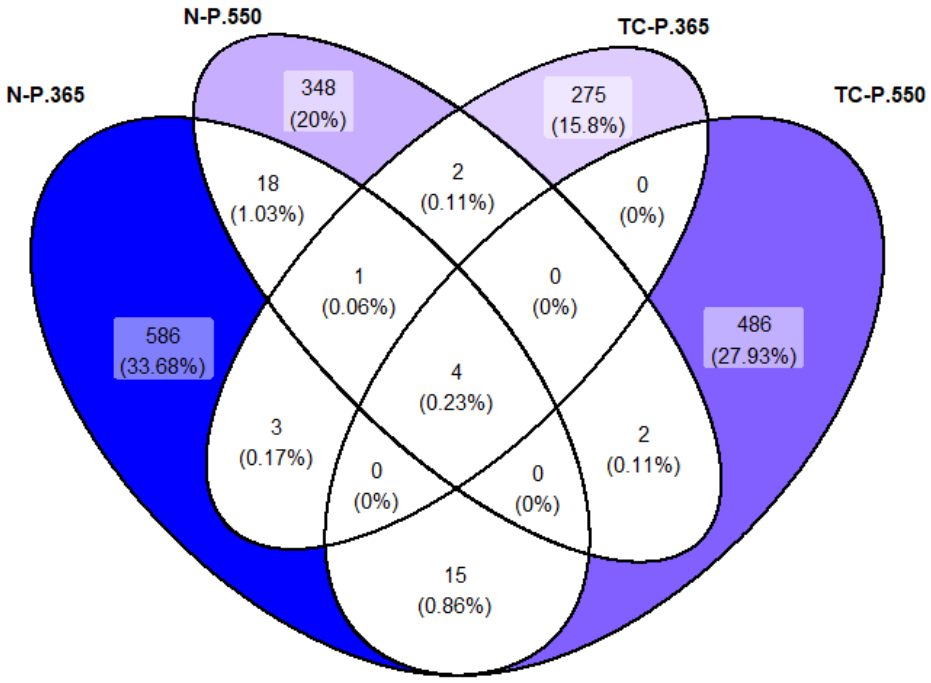

Figure 3.4. Venn-diagram with the number (and percentual) of genes associated with $TICK_{log}$ of yearling (P.365) and over yearling (P.550) Nellore (N) and Tropical Composite (TC) cattle.

Table 3.6. Results[1] of candidate gene prioritization analysis of genes associated with log-transformed tick counts shared between at least two genome-wide association studies

Gene ID	Included	Transcript Name	Data sets*	p-value
5S_Rrna	N	-	N+TC	-
7SK	N	-	N+TC	-
U6	N	-	N+TC	-
U1	N	-	N+TC	-
bta-mir-12003	N	-	N	-
C25H16orf72	N	-	N	-
bta-mir-12032	N	-	N	-
Metazoa_SRP	N	-	N+TC	-
SNORD36	N	-	N+TC	-
U2	N	-	N+TC	-
U4	N	-	N+TC	-
U5	N	-	N+TC	-
NTRK3	Y	neurotrophic receptor tyrosine kinase 3	N+TC	0.0003
LCP2	Y	lymphocyte cytosolic protein 2	N	0.0003
WWTR1	Y	WW domain containing transcription regulator 1	N+TC	0.002
CARHSP1	Y	calcium regulated heat stable protein 1	N	0.002
DOCK2	Y	dedicator of cytokinesis 2	N	0.002
CP	Y	Ceruloplasmin	N+TC	0.002
WNK1	Y	WNK lysine deficient protein kinase 1	N+TC	0.003
KCNMB1	Y	potassium calcium-activated channel subfamily M regulatory beta subunit 1	N	0.003
KCNIP1	Y	potassium voltage-gated channel interacting protein 1	N	0.004
JMJD1C	Y	jumonji domain containing 1C	N	0.005
CACNA1E	Y	calcium voltage-gated channel subunit alpha1 E	N+TC	0.005
PFN2	Y	profilin 2	N+TC	0.007
PMM2	Y	phosphomannomutase 2	N	0.02
TM4SF1	Y	transmembrane 4 L six family member 1	N+TC	0.03

USP7	Y	ubiquitin specific peptidase 7	N	0.04
ABAT	Y	4-aminobutyrate aminotransferase	N	0.06
RAD52	Y	RAD52 homolog, DNA repair protein	N+TC	0.07
HLTF	Y	helicase like transcription factor	N+TC	0.08
SLITRK5	Y	SLIT and NTRK like family member 5	N	0.13
RNF13	Y	ring finger protein 13	N+TC	0.13
HPS3	Y	HPS3 biogenesis of lysosomal organelles complex 2 subunit 1	N+TC	0.15
SLC44A1	Y	solute carrier family 44 member 1	N+TC	0.16
FOXI1	Y	forkhead box I1	N	0.21
TM4SF4	Y	transmembrane 4 L six family member 4	N+TC	0.23
OPCML	Y	opioid binding protein/cell adhesion molecule like	N+TC	0.25
REEP3	Y	receptor accessory protein 3	N	0.28
SPDL1	Y	spindle apparatus coiled-coil protein 1	N	0.30
TM4SF18	Y	transmembrane 4 L six family member 18	N+TC	0.52
SNORA70	Y	small nucleolar RNA, H/ACA box 70	N+TC	0.52
INSYN2B	Y	inhibitory synaptic factor family member 2B	N	0.57
COMMD2	Y	COMM domain containing 2	N+TC	0.61
TMEM186	Y	transmembrane protein 186	N	0.73
ANKUB1	Y	ankyrin repeat and ubiquitin domain containing 1	N+TC	0.73

*N = P.365 and/or P.550 for Nellore population, TC = P.365 and/or P.550 for Tropical Composite population

Eighteen genes were associated with P.365 and P.550 in Nellore, but without associations in the Tropical Composite population (Table 3.6). Out of those: 3 were discarded from the target gene list since no orthologous were found in *Homo sapiens*, furthermore, no transcripts were described for these genes yet; and 15 were kept in the target gene list.

The other 27 genes of Table 6 were associated with at least one data set of Nellore and one of Tropical Composite cattle. From these genes, 10 genes were discarded at ToppGene because no orthologous were found in *Homo sapiens*, and also no transcripts were described for them yet. The other 17 genes were included at the target gene list (Table 3.6).

The trained list was formed from the BIANA entries obtained at GUILDify [45]. The gene network that was associated to the provided keywords contained 3915 BIANA entries. The top-100 genes of these entries formed our training gene list (Table S9). At Table S10 are presented the different IDs recovered from the enrichment analysis for each training parameter based on the genes that were associated in at least two studied data sets, as well as the p-values used to evaluate if the function was enriched. Finally, from the 33 genes for which similarity scores were generated at prioritization analysis, 15 were prioritized for $TICK_{log}$ ($p<0.05$): 8 were associated with the trait only for Nellore cattle, and 7 were associated in the two studied breeds.

3.4. Discussion

3.4.1. Statistical models

3.4.1.1. Fixed effects

For both populations the contemporary group effects comprise different factors to be controlled due to metadata structure. However, they have the same purpose of grouping animals that were evaluated under similar conditions. For Nellore population we had more precise information regarding the group of animals that were raised at the same paddock. For instance, we had smaller groups that were submitted to much more similar conditions and evaluated for a single evaluator. For Tropical Composite, we did not have information about batches of animals being raised together, only the date of measurements, and the sex. Groups formed by the concatenation of these two factors were big, and much more different between each other, despite of being measured by different technicians. Other papers published with the Tropical Composite data set had already identified a significant effect of the counter on the $TICK_{log}$ [15]. For this reason, we included the counter effect to organize the contemporary groups of the Tropical Composite population.

For both studied populations, contemporary groups significantly explained part of the differences observed in $TICK_{log}$ between animals. Different grouping factors considered in the contemporary groups of each population, that are a result of the precision of the metadata information we had, might have contributed to the differences observed on the linear regression model's fit (R-squared).

As Tropical Composite is a crossbreed formed by the crossing of different Indicine and Taurine breeds, different Tropical Composite animals carry alleles that came from different subspecies, that might differ regarding parasite burden [18]. The differences observed in the genetic composition might be related with adaptation of tropical cattle

to challenging environmental conditions, not only related to parasite counts [30]. With this in mind, it is important to consider the genetic composition (%Zebu) as micro-environmental component that might affect $TICK_{log}$. Our results indicates that the higher the genetic composition derived from Indicine breeds, the lower is the observed tick counts in the animals. This negative relationship between $TICK_{log}$ and %Zebu is in evidence that *Bos taurus* and *Bos indicus* cattle differ regarding tick resistance, which agrees with previous published results [17,18,48].

3.4.1.2. Variance components

The animal's age does not influence on the genetic parameters for $TICK_{log}$. The mean interval between yearling and over yearling evaluations was approximately 6 months. Giglioti et al. [49] observed low to moderate repeatability for tick counts realized between 8-13, and 14-19 months old for Canchim cattle (0.06 and 0.20, respectively). The referred authors suggest that age might be one of the factors influencing the observed ticks counts across the intervals. For our populations, however, the age difference did not reflect on any differences in the genetic parameters. We believe that other factors as the single measures we used, allied with the effect of the season in which the measurements were made (that was not considered in the model) might have contributed to the absence of differences we observed.

In the opposite of age, the breed has significant effect on the genetic parameters estimates for $TICK_{log}$. The heritability for $TICK_{log}$ is low. Assuming that *Bos indicus* animals are more tick resistant [17], the low heritability can be a result of low environmental challenge provided by the low observed counts [50]. Similarly, low

heritability (0.15) were estimated for tick score of Brahman cattle [29], another *Bos indicus* breed. The heritability estimated for $TICK_{log}$ in Nellore cattle were lower than other recently published for the same population (0.12 [16] and 0.11 [6]). The observed differences are due to the reduced number of animals evaluated here (we only considered animals that had available genotypes) and the reduced number of observations since we only considered yearling or over yearling measurements while the referred authors used up to five measurements from 330 to 550 day old in average.

For the Tropical Composite population, heritability estimates were high. Similar results (h^2=0.37) were estimated for a population of mixed taurine breeds raised in Australia by Turner et al. [15]. For Brazilian populations of taurine or cross-bred (*Bos taurus* x *Bos indicus*) cattle, lower estimates were obtained for heritability of $TICK_{log}$ (from 0.17 to 0.25 for Hereford and Braford cattle [51,52], and 0.22 for Caracu cattle [53]). It is important to emphasize that, despite of the lower heritability observed for taurine or cross-bred cattle in Brazil, they were still higher than the estimates obtained for zebu animals. Thus, even considering that breeding management and population structure might be different in Australia and Brazil, and that this fact can interfere on the heritability for a same trait evaluated in both countries, there is still evidence that heritabilities are higher for taurine and cross-bred cattle than for Zebu animals. Another point that must be discussed is that, even for the Nellore population, where the heritability are low, it is possible to obtain genetic progress for $TICK_{log}$, as significant coefficients of additive genetic variance [54,55] were estimated for $TICK_{log}$ for both breeds and in both evaluated periods.

3.4.2. Genome wide association studies

Although we have found 1740 genes associated to at least one breed and in one period, we have considered that genes associations that were found only in one age, and for one breed, could easily represent false associations, as a result of high linkage disequilibrium with genes that truly affects resistance or statistic artefacts. Despite of that, 45 genes were associated with at least two data sets confirming previous information that $TICK_{log}$ is a polygenic trait [7]. Also, none of the 45 genes were associated with Tropical Composite cattle only, indicating that probably all the genes associated with resistance are being introduced by Zebu breeds [7], probably as part of the adaptive process to tropical environments [30].

Nine out of 27 genes associated with $TICK_{log}$ at both studied breeds were not submitted to prioritization analysis because no orthologous were found in *Homo sapiens*. They were all RNA genes. From the 18 genes that proceeded to prioritization analysis, 7 were prioritized: NTRK3, WWTR1, CP, WNK1, CACNA1E, PFN2, and TM4SF1. They are all protein coding genes. Up to now, there were no other studies focusing these genes as candidate for tick resistance.

Eighteen genes were associated with $TICK_{log}$ at the Nellore population only. The fact that we have some genes associated with $TICK_{log}$ in one studied breed, but not in the other, suggest that biological mechanisms that are necessary for the expression of the trait differ in the different studied breeds.

Out of the 18 genes associated with $TICK_{log}$ only, three (all RNA genes) were not considered for prioritization analysis, and 8 were prioritized ($p<0.05$): LCP2, CARHSP1, DOCK2, KCNMB1, KCNIP1, JMJD1C, PMM2, and USP7. LCP2 is a natural killer cell-mediated cytotoxicity gene that was recently associated with immune

response pathways differently expressed in sheep adapted to arid and heat environment, when compared to an exotic breed in China [56]. Tick resistance is a trait associated with adaption to tropical environments, that includes high temperatures and challenging environments, the association of LCP2 might indicate an adaptive evolution process stimulated by $TICK_{log}$.

DOCK2 is another gene that plays a role in immune cells, acting on both innate and adaptive immune cells [57]. This gene has been associated with environmental variance of litter size in rabbits (an indicator of animal's ability of quickly recover their performance in spite of environmental perturbations), and rabbits with low variance were found to be more resilient to infections [58]. The observed association might indicate the importance of immune mechanisms acting on the control of $TICK_{log}$ to the maintenance of a stable performance.

It is important to highlight that both enrichment and prioritization analysis are dynamic processes, and as new results of gene networks and gene expression studies are published, new genes can be prioritized, or new gene ontology terms can be associated with $TICK_{log}$ [6,59]. Also, a great proportion of RNA genes were not analyzed here, and thereafter were not discussed. However, we need to emphasize the importance of these RNA genes on regulatory processes, even when they are not codifying any protein, or their main action on the biological processes are not described yet [60].

3.5. Conclusions

It is possible to obtain genetic progress by selecting both Nellore and Tropical Composite breeds for tick resistance, although small progress should be expected per generation for Nellore cattle. The genetic mechanisms behind the studied phenotype changes across breeds, and Tropical Composite animals are more susceptible to tick infestations, the higher is the taurine composition of the animal. Genes associated to tick resistance in tropical composite cattle probably were inherited from the Bos indicus breeds that originated the cross-bred animals.

3.6. References

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3.7. Supplementary tables

The supplementary tables are presented in different sheets of the SupplementaryFile_Chapter2.xlsx

Table S1. SNPs with significant and suggestive association with log-transformed tick counts of yearling Nellore cattle

*σ^2_{SNP} is the variance for SNP solutions, used to calculate the p-values associated to each SNP

Table S2. SNPs with suggestive association with log-transformed tick counts of over yearling Nellore cattle

*σ^2_{SNP} is the variance for SNP solutions, used to calculate the p-values associated to each SNP

Table S3. SNPs with suggestive association with log-transformed tick counts of yearling Tropical Composite cattle

*σ^2_{SNP} is the variance for SNP solutions, used to calculate the p-values associated to each SNP

Table S4. SNPs with significant and suggestive association with log-transformed tick counts of over yearling Tropical Composite cattle

*σ^2_{SNP} is the variance for SNP solutions, used to calculate the p-values associated to each SNP

Table S5. Genes within 0.5Mbp downstream and upstream significant and suggestive SNPs associated with log-transformed tick counts of yearling Nellore cattle

Table S6. Genes within 0.5Mbp downstream and upstream suggestive SNPs associated with log-transformed tick counts of over yearling Nellore cattle

Table S7. Genes within 0.5Mbp downstream and upstream suggestive SNPs associated with log-transformed tick counts of yearling Tropical Composite cattle

Table S8. Genes within 0.5Mbp downstream and upstream significant and suggestive SNPs associated with log-transformed tick counts of over yearling Tropical Composite cattle

Table S9. Training gene list for tick resistance built at GUILDify using immunity, immune response, inflammation, ectoparasite, cytokines and tick as keywords

Table S10. Enrichment analysis realized for genes associated with $TICK_{log}$ in at least two of the evaluated data sets (P.365 of Nellore, P.550 of Nellore, P.365 of Tropical Composite, and P.550 of Tropical Composite Populations)

3.8. Próximos passos

Todas as análises e resultados apresentados aqui são resultados iniciais realizados para responder aos objetivos do artigo. Análises similares utilizando modelos bi

característicos serão realizadas na tentativa de aumentar a precisão nas estimativas dos parâmetros genéticos. Dessa forma em cada análise bi característica o número de animais incluídos na GRM será maior (todos os Nelore com dados em uma análise e todos os Tropical Composite com dados em outra).

Além disso, será analisada a possibilidade em se trabalhar com modelos de repetibilidade, como sugerido na literatura, ao invés da separação em arquivos de fenótipos ao ano e ao sobreano. Dessa forma será possível explorar um pouco mais os arquivos das suas populações, uma vez que temos de 1 a 5 medidas em Nelore, e de 1 a 3 em Tropical Composite.

CAPÍTULO 4 – CONSIDERAÇÕES FINAIS

A presente tese aborda diferentes aspectos da resposta a parasitose em bovinos criados em climas tropicais. Atualmente, poucos trabalhos têm como objetivo estudar a resiliência a fatores estressores em bovinos, e até onde sabemos, nenhum trabalho teve foco na caracterização da resiliência a diferentes parasitos. O segundo capítulo desta tese é, então, um estudo inicial em que se pretende propor uma metodologia para o estudo da resiliência a parasitos em uma população específica. Vale ressaltar que nessa população, que consideramos modelo para o estudo desse fenótipo manifestado a campo, temos alto controle sobre fatores ambientais que interferem na expressão tanto dos fenótipos de interesse produtivo, quando em relação às cargas parasitárias – ainda que baixas. Muitos questionamentos foram levantados a respeito do nível de infestação observados na propriedade em estudo, mesmo com baixos níveis de infestação nós

conseguimos demonstrar algumas características interessantes da resiliência a parasitos: a presença de variabilidade genética para esse fenótipo; a presença de interação genótipo x idade e o potencial efeito de diferentes mecanismos de resposta imune (inata x adquirida, humoral x celular) para justificar essa interação; e por fim, os diferentes mecanismos pelos quais os animais respondem à diferentes parasitos (carrapatos x nematódeos gastrointestinais x coccídeos).

Embora muitos trabalhos tenham sido realizados com os objetivos de se estimar parâmetros genéticos para resistência a carrapatos e entender os mecanismos biológicos envolvidos na expressão desse fenótipo como um todo, pouca ênfase foi dada nas diferenças observadas entre populações distintas, principalmente no que diz respeito aos diferentes mecanismos biológicos envolvidos na expressão de uma mesma característica em diferentes raças. O nosso objetivo com o trabalho apresentado no terceiro capítulo desta tese foi, por meio da combinação de avaliações genômicas, estudos de associação genômica ampla, análises de enriquecimento e análises de priorização de genes candidatos funcionais, estimar parâmetros genéticos e encontrar mecanismos associados a resistência em diferentes populações (Nelore x Tropical Composite). Conseguimos demonstrar que os parâmetros genéticos para as populações de Nelore e Tropical Composite diferem de forma significativa entre si. Ainda, conseguimos determinar que parte dessas diferenças podem ser justificadas pela diferença na composição racial (*Bos taurus* e *Bos indicus*) dos animais. Em relação às regiões com marcadores de efeito significativo sobre a contagem de carrapatos em escala logarítmica, podemos demonstrar que elas diferem em função da população estudada, e em função do arquivo de dados estudado (ano ou sobreano). Também é possível verificar a pouca sobreposição de genes associados em comum para indivíduos de raças diferentes, e

dentro de uma mesma raça em função das diferentes idades. Ainda não encontramos, entretanto, evidencias suficientes de que as diferenças observadas são devidas à idade. No que diz respeito às análises de enriquecimento funcional e genes priorizados, muitos genes e rotas biológicas foram recuperadas, entretanto, não encontramos genes em comum com aqueles já sabidamente associados à resistência. Nós acreditamos que esse panorama pode se alterar parcialmente na medida que novos estudos sejam publicados na comunidade científica.

Em resumo, todas as discussões levantadas aqui demonstram a dificuldade em se estudar resposta a parasitoses, dada a complexidade desses fenótipos e a dificuldade na mensuração adequada de dados de contagens de parasitos em larga escala. Ainda, é possível perceber que muitas perguntas precisam ser respondidas em relação à aplicabilidade das metodologias de avaliação da resposta a doença em populações diferentes e em diferentes estágios da vida dos animais. Embora exista potencial para obtenção de ganhos significativos em sanidade ao se incluir essas características enquanto critérios ou nos objetivos de seleção, muito ainda precisa ser entendido em relação à resposta a parasitoses antes que essa inclusão seja uma realidade nos programas de melhoramento genético.