UNIVERSIDADE ESTADUAL PAULISTA – UNESP
CÂMPUS DE JABOTICABAL

GENOMIC STUDIES OF *Babesia bigemina* AND *Anaplasma marginale* INFECTION LEVELS IN CATTLE

Andrea Renata da Silva Romero
Biotecnologista

2021

UNIVERSIDADE ESTADUAL PAULISTA – UNESP

CÂMPUS DE JABOTICABAL

GENOMIC STUDIES OF *Babesia bigemina* AND *Anaplasma marginale* INFECTION LEVELS IN CATTLE

Andrea Renata da Silva Romero

Orientador: Prof. Dr. Henrique Nunes de Oliveira

Coorientadores: Dra. Marcia Cristina de Sena Oliveira

Dr. Fernando Flores Cardoso

Tese apresentada à Faculdade de Ciências Agrárias e Veterinárias – UNESP, Câmpus de Jaboticabal, como parte das exigências para a obtenção do título de Doutora em Genética e Melhoramento Animal.

2021

R763g

Romero, Andrea Renata da Silva
Genomic studies of Babesia bigemina and Anaplasma marginale infection levels in cattle / Andrea Renata da Silva Romero. -- Jaboticabal, 2021
75 p.

Tese (doutorado) - Universidade Estadual Paulista (Unesp), Faculdade de Ciências Agrárias e Veterinárias, Jaboticabal
Orientador: Henrique Nunes de Oliveira
Coorientadora: Marcia Cristina de Sena Oliveira

1. Anaplasmose. 2. Babesiose. 3. Bovinos. 4. Genômica. 5. Polimorfismo de nucleotídeo único. I. Título.

Sistema de geração automática de fichas catalográficas da Unesp. Biblioteca da Faculdade de Ciências Agrárias e Veterinárias, Jaboticabal. Dados fornecidos pelo autor(a).

Essa ficha não pode ser modificada.

UNIVERSIDADE ESTADUAL PAULISTA

Câmpus de Jaboticabal

CERTIFICADO DE APROVAÇÃO

TÍTULO DA TESE: GENOMIC STUDIES OF *Babesia bigemina* AND *Anaplasma marginale* INFECTION LEVELS IN CATTLE

AUTORA: ÂNDREA RENATA DA SILVA ROMERO
ORIENTADOR: HENRIQUE NUNES DE OLIVEIRA
COORIENTADORA: MARCIA CRISTINA DE SENA OLIVEIRA
COORIENTADOR: FERNANDO FLORES CARDOSO

Aprovada como parte das exigências para obtenção do Título de Doutora em GENÉTICA E MELHORAMENTO ANIMAL, pela Comissão Examinadora:

Prof. Dr. HENRIQUE NUNES DE OLIVEIRA (Participaçao Virtual)
Departamento de Zootecnia / FCAV / Unesp - Jaboticabal

Prof. Dr. CEDRIC GONDRO (Participaçao Virtual)
Michigan State University / Michigan/EUA

Profa. Dra. LUCIA GALVÃO DE ALBUQUERQUE (Participaçao Virtual)
Departamento de Zootecnia / FCAV / Unesp - Jaboticabal

Pós-doutoranda CAMILA URBANO BRAZ (Participaçao Virtual)
Universidade de Wisconsin-Madison / Wisconsin/EUA

Pesquisador Dr. ROBERTO CARVALHEIRO (Participaçao Virtual)
Departamento de Zootecnia / FCAV / UNESP - Jaboticabal

Jaboticabal, 25 de fevereiro de 2021

DADOS CURRICULARES DO AUTOR

ANREA REANATA DA SILVA ROMERO – nascida na cidade de Corumbá – MS em 25 de setembro de 1993. Ingressou no curso de graduação em Biotecnologia em 2011 na Universidade Federal da Grande Dourados (UFGD), obtendo o título de Biotecnologista em março de 2015. Ingressou no curso de mestrado em Biologia Geral/Bioprospecção em março de 2015, na Faculdade de Ciências Biológicas e Ambientais da UFGD sob orientação Profa. Dra. Alexeia Barufatti e coorientação da Dra. Fabiane Siqueira. Durante o mestrado, foi bolsista da Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) realizando projeto em parceria com Embrapa Gado de Corte, obtendo título em fevereiro de 2017. Em 6 de março de 2017, ingressou no doutorado pelo Programa de Pós-graduação em Genética e Melhoramento Animal da Faculdade de Ciências Agrárias e Veterinárias da Universidade Estadual Paulista “Júlio de Mesquita Filho”, sob a orientação do Prof. Dr. Henrique Nunes de Oliveira. Iniciou o curso como bolsista da CAPES e passou a ser bolsista da Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) em 01 de maio de 2018. Realizou estágio “Sandwich” na Michigan State University, Estados Unidos, sob supervisão do Prof. Dr. Cedric Gondro pelo período de um ano, entre 2019 e 2020.

AGRADECIMENTOS

Agradeço à minha família, em especial minha mãe Iza por sempre se fazer presente em cada ligação, por acreditar em mim, e me apoiar em cada objetivo, mesmo que isso tenha significado conviver com a distância em nossa rotina. Agradeço também ao meu padrasto Marcelo Chaparro, irmãos Marcelinho e Livia e avó Ana por cada palavra e gesto de carinho que me fortaleceu ao longo de cada etapa.

Agradeço ao meu namorado André, pelo apoio nos momentos de dificuldade, pelo amor e carinho nos momentos de ansiedades, pelos sorrisos compartilhados ao longo da nossa jornada e também pela contribuição na elaboração deste trabalho.

Agradeço à cada colega que cruzou meu caminho, e que hoje tenho a alegria de chamá-los de amigos, pessoas com as quais tive a oportunidade de aprender e considero profissionais inspiradores, em especial, Daiane Becker, Diercles Cardoso, Danielly Beraldo, Camila Braz e André Mauric.

Agradeço ao professor Henrique Nunes de Oliveira, pelo voto de confiança em aceitar me orientar, pelos direcionamentos no decorrer do trabalho, por toda compreensão e paciência até o momento de conclusão do projeto.

Agradeço à Dra. Marcia Cristina de Sena Oliveira e Cíntia Hiromi Okino pela atenção e paciência dispensados para que eu pudesse obter os dados em meio ao caos da pandemia.

Ao professor Cedric por sempre ter estado de portas abertas para compartilhar seu conhecimento durante meu período na Michigan State University, à sua esposa Simone e sua equipe composta por Beatriz, Rodrigo, Hanna, Kenneth e Yasir, sempre dispostos a cooperar e contribuir uns com os outros. Agradeço aos amigos que fiz em East Lansing, com quem compartilhei momentos inesquecíveis e que levarei por toda minha vida.

Agradeço à Universidade Estadual Paulista – Júlio de Mesquita Filho pela oportunidade de cursar o doutorado no programa de Genética e Melhoramente Animal. Aos professores que investiram seu tempo e dedicação para compartilharem seus conhecimentos, proporcionando ensinamentos valiosos.

O presente trabalho foi realizado com apoio da Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Código de Financiamento 001.

À Fundação de Amparo à Pesquisa do Estado de São Paulo – FAPESP, pelo auxílio financeiro para desenvolvimento desta pesquisa (processo 2017/21000-0) e concessão da Bolsa Estágio de Pesquisa no Exterior (BEPE, processo 2019/00412-3).

SUMÁRIO

ESTUDOS GENÔMICOS DE NÍVEIS DE INFECÇÃO POR *Babesia bigemina* E *Anaplasma marginale* EM BOVINOS.

RESUMO – Carrapatos são responsáveis pela propagação de patógenos como a *Babesia bigemina* e *Anaplasma marginale* que causam as doenças babesiose e anaplamose bovina, respectivamente. A presença constante do carrapato nos rebanhos nacionais acarreta perdas econômicas e dificuldade na inclusão de raças taurinas, por serem animais mais susceptíveis a doenças parasitárias. Assim, o objetivo desse estudo foi estimar componentes de variância, acurácia de predição dos valores genéticos genômicos (GEBV), e realizar estudo de associação genômica ampla (GWAS) para os níveis de infecção da *B. bigemina* (BigIL) e *A. marginale* (AmIL). No capítulo dois foram feitas as análises para BigIL utilizando 1.882 animais (1.716 Braford e 166 Hereford) com genótipos imputados para painel de ~777.000 marcadores. Os componentes de variância foram obtidos por abordagem bayesiana. Para as estimativas de valores genéticos genômicos (GEBV) os fenótipos de contagem de carrapato (CC) e níveis de infecção por *B. bovis* (BovIL) foram incluídos no modelo multivariado visando ganhos em acurácia de predição da BigIL. As análises de predição genômica e GWAS foram realizadas utilizando o melhor preditor linear não viesado genômico (GBLUP). Foi observada baixa herdabilidade para BigIL (0,090) indicando maior influência ambiental do que genética aditiva. A estimativa da correlação genética entre BigIL e CC foi baixa (0.240), em contraste com a correlação entre BigIL e BovIL que foi alta (0,624). Os valores de correlação genética podem ter contribuído com aumento da acurácia quando comparamos o modelo univariado (0.246±0.189) e multivariado (0.606±0.180). No GWAS foi possível observar o a herança poligênica da BigIL. Nas regiões próximas dos SNP que explicaram maior parte da variância foram identificados 35 genes, os quais têm sido relacionados com resposta imune nos cromossomos 1, 4, 5, 7, 10, 13, 18, 20, 27, 29. No capítulo três foi realizada quantificação do número de cópias da *A. marginale* no sangue dos animais pela técnica de qPCR. Os componentes de variância foram estimados por análise Bayesiana e o método empregado para GWAS e estimação dos valores GEBV foi o melhor preditor linear não viesado genômico (GBLUP) usando modelo multivariado. As estimativas de herdabilidade e acurácia de predição foram baixas 0,090 e 0,180, respectivamente. A correlação genética entre AmIL com BigIL foi alta 0,793, enquanto as correlações entre AmIL com BovIL e contagem de carrapato foram próximas a zero. Ao explorar as top 10 regiões do GWAS, foram encontrados genes candidatos relacionados com interação entre hospedeiro e patógeno e resposta à inflamação, localizadas nos cromossomos 2, 5, 8, 10, 13, 15, 17, 20, 24, e 29. Foram identificadas sobreposições com QTL previamente descritos para susceptibilidade a tuberculose em bovinos e QTL com escore de células somáticas. Assim, os resultados obtidos no presente trabalho podem contribuir para estabelecimento de estratégias de seleção de animais que consigam manter níveis mais baixos de infecções por agentes causadores da Tristeza Parasitária Bovina.

Palavras-chave: Anaplasmose, babesiose, bovinos, genômica, polimorfismo de nucleotídeo único,

GENOMIC STUDIES OF *Babesia bigemina* AND *Anaplasma marginale* INFECTION LEVELS IN CATTLE

ABSTRACT – Ticks are responsible for the spread of pathogens such as *Babesia bigemina* and *Anaplasma marginale* that cause the diseases babesiosis and anaplamosis, respectively. The constant presence of the tick in national herds leads to economic losses and difficulty in the inclusion of taurine breeds, as they are animals more susceptible to parasitic diseases than indicine cattle. Thus, the objective of this study was to estimate components of variance, accuracy of prediction, and to conduct a genome-wide association study (GWAS) for the levels of infection of *B. bigemina* (BigIL) and *A. marginale* (AmIL). In chapter two, analyses for BigIL were performed using 1,882 animals (1,716 Braford and 166 Hereford) with genotypes imputed to a panel of ~777,000 markers. The variance components were obtained using the Bayesian approach. For the estimation of genomic breeding values (GEBV), tick count (CC) phenotype and levels of *B. bovis* infection (BovIL) were included in the multivariate model aiming at gains in BigIL prediction accuracy. Genomic prediction and GWAS analyses were performed using genomic best linear unbiased prediction method (GBLUP). Low heritability was observed for BigIL (0.090) indicating greater environmental influence than genetic. The estimate of the genetic correlation between BigIL and CC was low (0.240), in contrast to the correlation between BigIL and BovIL which was high (0.624). The genetic correlation values may have contributed to an increase in accuracy when comparing the univariate (0.246±0.189) and multivariate (0.606±0.180) models. GWAS results showed a polygenic inheritance of BigIL. In the regions close to the SNPs that explained most of the additive variance were identified 35 genes previously related to the immune response located on chromosomes 1, 4, 5, 7, 10, 13, 18, 20, 27, 29. In chapter three, the variance components were estimated by Bayesian analysis and the method used for GWAS and estimation of the values was the best linear biased genomic predictor (GBLUP) using the multivariate model. The estimates of heritability and prediction accuracy were 0.090 and 0.180, respectively. The genetic correlation between AmIL and BigIL was high 0.793, while the correlations between AmIL and BovIL and tick count were close to zero. Exploring the top 10 regions of GWAS, candidate genes were found related to host-pathogen interaction and response to inflammation, located on chromosomes 2, 5, 8, 10, 13, 15, 17, 20, 24, and 29, overlapping with QTL previously described for susceptibility to tuberculosis in cattle and QTL with somatic cell score were identified. Thus, the results obtained in the present work can contribute to the establishment of selection strategies that result in animals that are able to maintain lower levels of infections by agents that cause Bovine Parasitic Sadness.

Keywords: Anaplasmosis, babesiosis, cattle, genomic, single nucleotide polymorphisms

CAPÍTULO 1 - Considerações gerais

1.1. Introdução

A pecuária bovina é responsável pela arrecadação de 110 bilhões de reais (valor bruto estimado em agosto de 2020), que equivale a aproximadamente 43% dos ganhos com agropecuária (Mapa, 2020). Animais zebuínos, em especial da raça Nelore, representam 80% do total de ~213 milhões cabeças de gado no território nacional. A predominância de animais zebuínos no Brasil se deve a maior capacidade adaptativa às condições ambientais, comparados aos taurinos (Menezes, 2016; Mapa, 2020).

Embora bovinos da raça Hereford sejam considerados melhores adaptados a baixas temperaturas, é uma das raças taurinas utilizadas para corte e cruzamentos no Brasil. Animais desta raça possuem características relacionadas com qualidade de carne que são desejáveis para os produtores e estão presentes predominantemente no sul do país, por ser região de clima ameno. Animais cruzados, como é o caso do Braford (produto do cruzamento entre Hereford e Nelore), são produzidos com intuito de explorar a heterose e contornar problemas com adaptação a temperatura e parasitas, visto que o carrapato, por exemplo, e as doenças por ele transmitidas estão presentes em praticamente todo o território nacional (Alencar, 2004).

Perdas econômicas globais em torno de 18 bilhões de dólares foram atribuídas às doenças transmitidas por carrapatos, entre elas estão a babesiose e anaplasmose bovina (Porto-Neto et al., 2011). A babesiose é causada pelos protozoários *Babesia bovis* e *Babesia bigemina* enquanto a anaplasmose é causada pela riquétsia *Anaplasma marginale.* Esses patógenos podem ser transmitidos simultaneamente aos bovinos junto à saliva na sucção sanguínea durante a alimentação do *Boophilus microplus*, causando um complexo parasitário denominado Tristeza Parasitária Bovina, TPB (Kessler, 2001). O modo de ação dos patógenos são semelhantes, causando danos aos eritrócitos. Animais com casos clínicos da doença, apresentam como principal sintoma quadros graves de anemia. Embora exista vacina trivalente consideradas eficazes com patógenos atenuados da *B. bovis*, *B. bigemina* e *A. centrale*, há

ocorrências de reações severas à vacina, com conversão para estágio clínico da doença (Kessler et al., 1998; Dantas-Torres; Otranto, 2017).

No Brasil o controle estratégico do carrapato (vetor da anaplasmose e babesiose) é uma medida de controle de surtos, pois animais jovens possuem maior resistência à forma grave da doença, devido a mecanismos que envolvem anticorpos colostrais e imunidade inata. Nesse caso, os bezerros possuem rápida recuperação e se tornam portadores persistentes dos patógenos. Regiões em que há presença do carrapato em todas as estações, é estabelecida a estabilidade enzoótica, que gera uma constante contaminação dos animais jovens, impedindo que o primeiro contanto ocorra apenas quando adultos, quando são mais susceptíveis à fase aguda da doença (Mahoney, 1969; Mahoney; Ross, 1972; Buling et al., 2007).

Um dos desafios na implantação de programa de seleção para animais resistentes à babesiose e anaplasmose bovina, está no estabelecimento de estratégias para se avaliar a susceptibilidade dos animais. Nesse sentido, o avanço de técnicas moleculares como reação em cadeia da polimerase quantitativa (qPCR) possibilitou obter um fenótipo quantitativo que permitisse classificar os animais como mais resistentes ou susceptíveis à patógenos (Pereira, 2018). O uso do fenótipo quantitativo em estudos genômicos podem contribuir para a identificação de genes e regiões genômicas associadas com características de resistência a *B. bigemina* e *A. marginale*, levando ao conhecimento dos mecanismos biológicos e fisiológicos envolvidos na manifestação desta doença.

1.2. Objetivos

O objetivo desse estudo foi estimar parâmetros genéticos, acurácia de predição dos valores genéticos genômicos (GEBV), bem como realizar estudo de associação genômica ampla para os níveis de infecção da *Babesia bigemina* (BigIL) e *Anaplasma marginale* (AmIL).

1.3. Revisão de literatura

A pecuária desempenha papel de relevância para economia brasileira, representando arrecadação de 252,27 bilhões de reais até agosto de 2020, destes, 110 bilhões de reais equivalem a produção de bovinos, constituída por mais de 213 milhões cabeças (MAPA, 2020). Devido a melhor adaptação dos animais zebuínos (*Bos taurus indicus ou Bos primigenius indicus*) às condições tropicais, estes correspondem a 80% do rebanho nacional, composto em sua maioria por animais da raça Nelore (Menezes, 2016). Em contraste às características adaptativas de animais *B. indicus*, os taurinos (*Bos taurus taurus ou Bos primigenius taurus*) possuem melhor desempenho produtivo e reprodutivo quando estão em ambiente favorável (Jonsson; Bock; Jorgensen, 2008; Ibelli et al., 2012).

A raça inglesa Hereford foi introduzida no Brasil em 1.858 visando aumentar a produtividade e qualidade da carne. No entanto, estes animais apresentam problemas de adaptação em regiões com baixa qualidade do pasto, presença de parasitas e alta incidência luminosa que resultam em frequente ocorrência de doenças como carcinoma ocular. Com isso, o cruzamento entre Hereford e Nelore foi adotado para formação da raça Braford, buscando explorar a heterose e contornar problemas adaptativos (Leal, 2009).

Aspectos climáticos contribuíram para que houvesse maior concentração de animais Hereford no Sul do país, onde a temperatura é mais amena. Por outro lado, a raça Braford está disseminada nas regiões Norte, Centro-Oeste e Sul (Bertipaglia et al., 2008; Leal, 2009). Além da temperatura, outra limitação para uso dos taurinos é a presença constante do carrapato e das doenças por ele transmitidas em praticamente todo o território nacional, como é o caso dos patógenos que provocam a tristeza parasitária bovina, TPB (De Alencar, 2004).

A TPB é um complexo de doenças causadas por protozoários e riquétsias dos gêneros *Babesia* e *Anaplasma*, respectivamente. Na América do Sul, África e Austrália, os agentes da babesiose são *B. bovis* e *B. bigemina* e da anaplamose a *A. marginale*, ambas enfermidades possuem o carrapato (*B. microplus)* como principal vetor biológico da doença (Kessler; Schenk, 1998). Os carrapatos são atraídos ao seu hospedeiro por estímulos como, visuais, temperatura e odor. As barreiras físicas que afetam a resistência ao carrapato

incluem comprimento e pigmentação da pelagem (clara ou escura), vibração da pele e/ou capacidade de autolimpeza (Tabor et al., 2017).

O *Rhipicephalus microplus* é parasita preferencial de bovinos, no entanto, é capaz de completar a fase parasitária em espécies como, búfalos, cabras, ovelhas, cavalos, porcos, cães e alguns roedores, com menor taxa de sucesso na postura dos ovos (Ma et al., 2016; Obregón et al., 2020). Apesar de serem considerados parasitas de único hospedeiro, o carrapato pode se deslocar de um bovino para outro ao buscar novo local de fixação para completar a ecdise e reiniciar sua alimentação. A troca de hospedeiro é facilitada pelo contato físico entre bovinos, principalmente durante a amamentação ou entre animais em atividade reprodutiva (Kessler, 2001).

O ciclo de vida dos carrapatos inclui fases de vida livre e parasitária, as fêmeas totalmente ingurgitadas (cheias de sangue) se desprendem do hospedeiro para liberar os ovos no solo, nesse momento se inicia a primeira fase de vida livre do carrapato com o período de pré-postura. Na segunda fase de vida livre ocorre a postura e posterior eclosão dos ovos. A parte parasitária de seu ciclo de vida é constituída pelas fases de larvas, ninfas e adultos, sendo hematófagos nos três estágios (Morand; Beaudeau; Cabaret, 2011). Umidade e temperatura são fatores determinantes para o sucesso das eclosões. Temperaturas entre 25 a 33°C são consideradas favoráveis, mas as taxas de eclosões são maiores do que 95% em temperaturas entre 28 a 33°C (Sutherst; Bourne, 2006).

O *Rhipicephalus microplus* é capaz de transmitir diversos patógenos em momentos diferentes do seu ciclo de vida. No caso da *Babesia* spp., as fêmeas desempenham papel de maior relevância para a transmissão do protozoário devido sua capacidade de transmitir o vetor para seus descendentes, visto que o *Rhipicephalus microplus* é capaz de transmitir *B. bovis* para os bovinos apenas em sua fase larval e *B. bigemina* em sua fase de ninfa. Na transmissão da *A. marginale* os *Rhipicephalus microplus* machos desempenham papel fundamental por terem maior mobilidade e longevidade, ocorrendo transferência do carrapato de um hospedeiro infectado, para outro (Dalgliesh; Stewart, 1983; Kessler, 2001).

A TPB é caracterizada pelo fenômeno denominado por Goff et al. (2001) de resistência inversa em função da idade, no qual animais jovens são mais

resistentes do que os adultos. Outra característica importante destas enfermidades é a persistência, ou seja, uma vez infectado e após a fase aguda da doença, o hospedeiro permanece infectado por longos períodos e eventualmente ao longo de toda vida.

Em regiões em que as condições climáticas permitem a sobrevivência do carrapato durante todo o ano, os bovinos são infectados com os hemoparasitas quando jovens, momento o qual são mais resistentes. Os bezerros estabelecem então uma sólida imunidade contra a doença ao se recuperarem, mas não contra os agentes da TPB, podendo permanecer com infecção persistente (Allred, 2003). Os bovinos destas regiões atuam como portadores sadios e contaminando outros carrapatos. Assim, fica estabelecido um equilíbrio, onde os bovinos jovens são infectados e permanecem imunizados como portadores sadios durante toda a vida. Áreas em que esta situação ocorre são caracterizadas como em estabilidade enzoótica (Mahoney, 1969; Mahoney; Ross, 1972). Por outro lado, regiões com baixa temperatura no inverno, inferior à 15°C, favorecem a redução drástica na quantidade de carrapatos, diminuindo os níveis de imunidade do rebanho. Nessa situação, graves surtos de TPB podem ocorrer ocasionalmente com altas taxas de mortalidade. Mesmo em regiões onde há estabilidade enzoótica bem estabelecida, o manejo inadequado no controle do carrapato pode interromper esta condição (Farias, 1995; Chagas; Furlong; Nascimento, 2001).

1.3.1. *Babesia bigemina*

Babes (1888) detectou microrganismos nos eritrócitos de bovinos portadores da doença hemoglobinúria enzoótica bovina, que embora fosse um protozoário, foi classificado a princípio como uma bactéria (*Haematococcus bovis*). Smith; Kilborne (1893) associaram a Febre do Texas com a hemoglobinúria enzoótica bovina, provocada pelo protozoário que denominaram *Pyrosoma bigemina*, sendo este, o primeiro relato de um protozoário transmitido por artrópode. No mesmo ano, devido a similaridade entre os microorganismos, (Starcovici, 1893) propôs a fusão do *Haematococcus bovis* e *Pyrosoma bigemina* para o gênero *Babesia*. Em 1923 foi descrito pela primeira vez o desenvolvimento da *Babesia* no carrapato *R. microplus* (Bock et al., 2004).

A infecção das fêmeas *R. microplus* depende de fatores como grau de parasitemia dos eritrócitos bovinos, temperatura e umidade (Riek, 1964). Hospedeiros vertebrados naturalmente infectados pela *B. bigemina* infectam mais de 90% dos carrapatos, que são capazes de transmitir o protozoário para mais da metade da progênie larval. A temperatura exerce influência na taxa de infecção do carrapato adulto, tendo seu desenvolvimento inibido em temperaturas abaixo de 20°C, mas com desenvolvimento completo aos 28°C. No entanto, as baixas temperaturas não afetam o desenvolvimento do protozoário na fase larval do *Rhipicephalus microplus* (Riek, 1964).

Embora existam muitas formas do protozoário, durante o ciclo de vida nos eritrócitos bovinos, apenas a forma sexual madura (gametócitos) são capazes de se desenvolver no hospedeiro invertebrado. Ao passo que as formas assexuadas que constituem maior proporção nos eritrócitos perecem no período de 24 horas após a ingestão do carrapato de sangue infectado, ficando viáveis apenas os protozoários nas formas ovais e esféricas (Riek, 1964).

A detecção da *Babesia* spp. no carrapato pode ser observada após 10 horas do início da alimentação do sangue infectado e, aproximadamente 50 horas depois ocorre a formação de novas organelas envolvidas na fusão dos gametas. Oitenta horas após o início da alimentação, o zigoto resultante invade células epiteliais do intestino do carrapato e, posteriormente, se move para ácinos salivares via hemolinfa. Nas glândulas salivares dos carrapatos formam um esporoblasto multinucleado que preenche a célula hospedeira. O protozoário também invade ovários e ovos do carrapato, caracterizando a transmissão transovariana. Durante o início da segunda alimentação do carrapato ocorre o desenvolvimento de organelas especializadas para formação do esporozoíto maduro. Cerca de 5.000 a 10.000 esporozoítos podem ser produzidos dentro de um esporoblasto (Homer et al., 2000).

No hospedeiro vertebrado, apenas os eritrócitos são invadidos pelo parasita, por meio de moléculas na superfície do protozoário reconhecidas por receptores das células que facilitam a entrada. O período de incubação da doença é de 12 dias e os sintomas da fase aguda incluem anemia, febre, hemoglobinúria (hemoglobina na urina), ataxia (sintoma que afeta coordenação dos movimentos) e em alguns casos pode levar a morte do animal (Bock et al., 2004; Barreira et al., 2005).

No interior dos eritrócitos, ocorre a reprodução assexuada da *Babesia* spp, que adquire uma forma cíclica e posteriormente se desenvolve para a forma de merozoítos replicáveis. Aspectos morfológicos da *Babesia* spp. permite sua classificação em pequena babesia (1,0 a 2,5 µm de comprimento) como a *B. bovis* e grande babesia (2,5 a 5,0 µm de comprimento) como a *B. bigemina*. Após a divisão dos merozoítos, ocorre a lise dos eritrócitos e os protozoários migram para novas células (Ganzinelli et al., 2018).

A lise dos glóbulos vermelhos e consequente anemia em bovinos infectados por *B. bigemina* foram relacionadas a danos oxidativos na membrana celular, que são ricas em ácidos graxos poli-insaturados (alvo primário de radicais livres). Os radicais livres possuem vida curta e são de difícil detecção, por isso, produtos da peroxidação lipídica são utilizados como indicadores do estresse oxidativo in vivo, como o malondialdeído (MDA) e metemoglobina (MetHb) (Esterbauer, 1996; Cipierre et al., 2013), ambos correlacionados com o nível de parasitemia em bovinos (0,65 e 0,75, respectivamente). Além disso, o aumento da parasitemia está relacionado com a fragilidade osmótica por distúrbios na membrana dos eritrócitos. Esses processos são similares ao que ocorre em animais infectados por *B. bovis* (Saleh, 2009).

A *B. bigemina* também atua na redução da disponibilidade de oligoelementos como cobre, zinco, manganês e selênio, que compõem enzimas antioxidantes, aumentando a susceptibilidade dos eritrócitos à danos oxidativos. Este fenômeno também ocorre durante a infecção por *A. marginale* (Esmaeilnejad et al., 2020).

1.3.2. *Anaplasma marginale*

Anaplasmose é uma doença hemolítica causada pela riquétsia *A. marginale.* Theiler (1910) identificou a presença de pontos marginais nos eritrócitos bovinos ao investigar o sangue de animais debilitados por anemia aguda. O patógeno foi classificado primeiramente como protozoário, mas posteriormente foi reclassificado como riquétsia pertencente ao gênero *Anaplasma* (Dumler et al., 2001). Embora moscas hematófagas sejam conhecidas como transmissoras da doença, no Brasil o principal vetor biológico da doença é o *R. microplus* (Ruiz; Passos; Ribeiro, 2005).

Em contraste com *Babesia* spp. a transmissão transovariana não é um fator relevante na disseminação da *A. marginale* e não há consenso de que realmente ocorra. A predominância de larvas não infectadas ocorre porque, em temperaturas favoráveis (28 a 33°C), o carrapato oviposita antes da riquétsia ter se instalado nos ovários das fêmeas. Ainda que o tempo de incubação das fêmeas ingurgitadas seja elevado devido à baixa temperatura, existem poucos relatos de que esse tipo de transmissão ocorra, e quando ocorrem afetam pequenas porcentagens dos descendentes (Ribeiro; Lima, 1995; Moura et al., 2003; Esteves et al., 2015).

O ciclo de desenvolvimento da *A. marginale* é similar ao descrito para *Babesia* spp. O patógeno se instala preferencialmente em células epiteliais do intestino do hospedeiro (carrapato) e apresenta duas fases de desenvolvimento. Na primeira fase, o patógeno se divide por fissão binária formando colônias. A segunda fase de desenvolvimento da *A. marginale* constitui sua forma infectante, em que ocorre a migração para demais tecidos como glândulas salivares. Uma vez instaladas nas glândulas salivares, a riquétsia é transmitida para os bovinos, junto a saliva, durante a alimentação do carrapato (Ribeiro; Lima, 1996; Kocan et al., 2004).

Após a infecção, a *A. marginale* se instala nos eritrócitos bovinos, e formam corpos iniciais constituídos de 4 a 8 riquétsias, ligados à membrana. Os patógenos são reconhecidos por células reticuloendoteliais que fagocitam os eritrócitos infectados (reconhecidas como patógeno) o que pode causar a diminuição das células vermelhas acarretando em quadros de anemia. Bovinos infectados podem permanecer assintomáticos em média de 28 dias, após o período pré-patente, os sinais clínicos podem incluir anemia, icterícia sem hemoglobinemia (hemoglobina no plasma) ou hemoglobinúria, além disso, febre, perda de peso, aborto, letargia, icterícia e, frequentemente, morte de animais com mais de 2 anos de idade (Kocan et al., 2010).

Assim como a babesiose, a anaplasmose bovina causa a diminuição de enzimas, oligoelementos antioxidantes e aumento da peroxidação lipídica dos eritrócitos com consequente aumento da susceptibilidade dessas células (Esmaeilnejad et al., 2018).

1.3.3. Controle das doenças

Medidas adotadas para prevenção de surtos da babesiose e anaplasmose incluem o controle do vetor, quimioprofilaxia e uso de vacinas. No Brasil é encorajado o controle estratégico do carrapato, visando estabilidade enzoótica com infecção recorrente dos animais. No entanto, o uso indiscriminado de métodos químicos de controle colaborou para o surgimento de parasitas resistentes. Além disto, os riscos de contaminação ambiental e à saúde pública associada ao uso destes produtos químicos são consideráveis (Piper et al., 2008; Ibelli et al., 2012).

A quimioprofilaxia se baseia no uso de antibióticos como a tetraciclina e imidocarb para controle da anaplasmose e babesiose, respectivamente. O tratamento permite a recuperação completa do animal, que apresenta apenas sinais brandos da doença, porém podem levar outros patógenos a desenvolverem resistência a antibióticos, como *Escherichia coli* (Hirsh et al., 1974; Gonçalves, 2000).

No Brasil, há dois tipos de vacinas disponíveis comercialmente para prevenção de babesiose e anaplasmose bovina, ambas se baseiam no uso trivalente dos patógenos atenuados (*Anaplasma centrale*, *B. bovis* e *B. bigemina*), sendo *A. centrale* utilizada contra *A. marginale*. A principal diferença entre as vacinas é a forma de armazenamento, sendo que a forma congelada pode ser conservada no gelo seco por longos períodos enquanto a forma refrigerada deve ser usada dentro de sete dias. Apesar de apresentarem resultados satisfatórios na imunização do gado, há casos de reações severas à vacina e conversão para o caso clínico da doença (Kessler et al., 1998; Dantas-Torres; Otranto, 2017).

Vacinas recombinantes que se baseiam na resposta imune do carrapato e dos bovinos tem sido propostas, tanto para babesiose como para anaplasmose bovina, porém até o momento não houve consolidação de nenhuma das metodologias (Ortega-Sánchez et al., 2020; Sarli et al., 2020). Dessa forma, a resistência natural do hospedeiro às infecções pode ser um método viável de controle de doenças parasitárias.

1.3.4. Quantificação dos níveis de infecção por *A. marginale* e *B. bigemina*

Técnicas como ELISA, análise de esfregaços sanguíneos e reação da cadeia polimerase (PCR) convencional podem ser utilizadas no diagnóstico da babesiose e anaplasmose bovina. No entanto, a técnica de PCR em tempo real agregou ao diagnóstico, também a possibilidade de obter um fenótipo quantitativo que permitisse classificar os animais como mais resistentes ou susceptíveis. A técnica é considerada sensível, específica e de rápido diagnóstico (Buling et al., 2007).

A qPCR consite no monitoramento da amplificação em tempo real que ocorre por meio de sinais fluorescentes emitidos pelas moléculas associadas aos *amplicons*. Posteriormente, com auxílio de um software específico, os sinais luminosos que correspondem aos produtos gerados são convertidos em valor numérico. Para quantificação do DNA e comparação com outras amostras, é determinado um ciclo dentro da fase exponencial, que é denominado ciclo de quantificação, Cq (Pereira, 2018). Considerando que a florescência emitida é proporcional a quantidade de DNA obtida na amostra, os valores de Cq podem ser considerados quantificações absolutas. Nesse caso, as quantificações se baseiam em uma curva padrão construída a partir da leitura de amostras com número de cópias conhecidas do DNA alvo (Pereira, 2018). Alguns estudos como Bilhassi et al., (2014), e Buling et al., (2007) têm demonstrado a eficiência da técnica nas quantificações absolutas de patógenos como *B. bovis*, *B. bigemina* e *A. marginale*.

1.3.5. Estudos genômicos

Apesar do *Rhipicephalus microplus* ser o principal transmissor da *B. bigemina* e *A. marginale*, pesquisas têm demonstrado não haver correlação entre contagem de carrapatos e número de cópia da *B. bovis*, *B. bigemina* e *A. marginale*. Isto sugere que a seleção direta para resistência aos patógenos seja mais indicada do que a seleção para resistência ao carrapato (Giglioti et al., 2017; Martins et al., 2020).

Estudos genômicos têm sido realizados na busca por animais resistentes à agentes parasitários como *B. bovis* (Cavani et al., 2020), *Mycobacterium bovis* (tuberculose bovina) (Richardson et al., 2016) e *Staphylococcus aureus* (mastite)

(Freebern et al., 2020). Esses estudos têm demonstrado que o uso de informações genômicas para o melhoramento genético pode acelerar a resposta à seleção, assim como identificar regiões envolvidas na variação da expressão de características de interesse econômico. Dentre as ferramentas utilizadas para estas finalidades, o estudo de associação genômica ampla (GWAS) e a seleção genômica (GWS) têm sido considerados importantes no aprimoramento das técnicas de melhoramento genético (Van Eenennaam et al., 2014).

O GWAS é um método de mapeamento genético que tem o objetivo de identificar marcadores moleculares em desequilíbrio de ligação com Loci de características quantitativas (QTL) de fenótipos de interesse e genes candidatos. Informações obtidas com GWAS podem ser utilizadas em estudos de GWS, após validadas. Além disso, o GWAS é uma abordagem que permite uma melhor compreensão da participação de genes em vias metabólicas envolvidas em processos biológicos que afetam características de interesse econômico (Cantor; Lange; Sinsheimer, 2010; Ku et al., 2010).

A GWS busca predizer desempenho futuro com base em subconjuntos de SNP, podendo auxiliar na identificação e seleção de animais com valor genético superior, bem como na diminuição do intervalo entre gerações (Biegelmeyer et al., 2016). A GWS tem mostrado bons resultados, principalmente para características de difícil mensuração, como aquelas que são expressas em apenas um dos sexos ou observada apenas após o abate, e aquelas relacionadas à resistência de doenças ou de baixa herdabilidade (Hayes; Goddard, 2001).

1.4. Referências

Allred DR (2003) Babesiosis: persistence in the face of adversity. **Trends in Parasitology**, 19:51–55.

Babes V (1888) Sur l'hemoglobinurie bacterienne du boeuf. **CR Acad. Sci**, 107:692–694.

Barreira JD, Rossi MID, DA Silva GILVO, Pires FA, Massard CL (2005) caracterização morfológica e aspectos biológicos das formas evolutivas de Babesia bigemina (SMITH; KILBORNE, 1893)(PROTOZOA: BABESIIDAE) EM Boophilus microplus (CANESTRINI, 1887). **Revista Brasileira de Parasitologia Veterinária**, 14:1–6.

Bertipaglia ECA, Silva RG da, Cardoso V, Fries LA (2008) Desempenho

reprodutivo, características do pelame e taxa de sudação em vacas da raça Braford. **Revista Brasileira de Zootecnia**, 37:1573–1583.

Biegelmeyer P, Gulias-Gomes CC, Caetano AR, Steibel JP, Cardoso FF (2016) Linkage disequilibrium, persistence of phase and effective population size estimates in Hereford and Braford cattle. **BMC genetics**, 17:32.

Bilhassi TB, Oliveira HN, Ibelli AMG, Giglioti R, Regitano LCA, Oliveira-Sequeira TCG, Bressani FA, Malagó Jr W, Resende FD, Oliveira MCS (2014) Quantitative study of Babesia bovis infection in beef cattle from São Paulo state, Brazil. **Ticks and tick-borne diseases**, 5:234–238.

Bock R, Jackson L, De Vos A, Jorgensen W (2004) Babesiosis of cattle. **Parasitology**, 129:S247–S269.

Buling A, Criado-Fornelio A, Asenzo G, Benitez D, Barba-Carretero JC, Florin-Christensen M (2007) A quantitative PCR assay for the detection and quantification of Babesia bovis and B. bigemina. **Veterinary parasitology**, 147:16–25.

Cantor RM, Lange K, Sinsheimer JS (2010) Prioritizing GWAS results: a review of statistical methods and recommendations for their application. **The American Journal of Human Genetics**, 86:6–22.

Cavani L, Braz CU, Giglioti R, Okino CH, Gulias-Gomes CC, Caetano AR, De Sena Oliveira MC, Cardoso FF, De Oliveira HN (2020) Genomic study of Babesia bovis infection level and its association with tick count in Hereford and Braford cattle. **Frontiers in Immunology**, 11.

Chagas AC de S, Furlong J, Nascimento CB (2001) Comportamento e ecologia de fêmeas ingurgitadas do carrapato Boophilus microplus em pastagem de Brachiaria decumbens no Brasil. **Brazilian Journal of Veterinary Research and Animal Science**, 38:188–191.

Cipierre C, Haÿs S, Maucort-Boulch D, Steghens J-P, Picaud J-C (2013) Malondialdehyde adduct to hemoglobin: a new marker of oxidative stress suitable for full-term and preterm neonates. **Oxidative medicine and cellular longevity**, 2013.

Dalgliesh RJ, Stewart NP (1983) The use of tick transmission by Boophilus microplus to isolate pure strains of Babesia bovis, Babesia bigemina and Anaplasma marginale from cattle with mixed infections. **Veterinary Parasitology**, 13:317–323.

Dantas-Torres F, Otranto D (2017) Anaplasmosis. In: MARCONDES, C. B. (Ed.). **Arthropod Borne Diseases**. Cham: Springer International Publishing, p. 215–222.

De Alencar MM (2004) Perspectivas para o melhoramento genético de bovinos de corte no Brasil.

Dumler JS, Barbet AF, Bekker CP, Dasch GA, Palmer GH, Ray SC, Rikihisa Y,

Rurangirwa FR (2001) Reorganization of genera in the families Rickettsiaceae and Anaplasmataceae in the order Rickettsiales: unification of some species of Ehrlichia with Anaplasma, Cowdria with Ehrlichia and Ehrlichia with Neorickettsia, descriptions of six new species combi. **International journal of systematic and evolutionary microbiology**, 51:2145–2165.

Esmaeilnejad B, Tavassoli M, Samiei A, Hajipour N, Imani-Baran A, Farhang-Pajuh F (2018) Evaluation of oxidative stress and antioxidant status, serum trace mineral levels and cholinesterases activity in cattle infected with Anaplasma marginale. **Microbial pathogenesis**, 123:402–409.

Esmaeilnejad B, Tavassoli M, Dalir-Naghadeh B, Samiei A, Rajabi S, Mohammadi V, Anassori E, Ehteshamfar S (2020) Status of oxidative stress, trace elements, sialic acid and cholinesterase activity in cattle naturally infected with Babesia bigemina. **Comparative Immunology, Microbiology and Infectious Diseases**, 101503.

Esterbauer H (1996) Estimation of peroxidative damage. A critical review. **Pathologie-biologie**, 44:25–28.

Esteves E, Pohl PC, Klafke GM, Reck J, Fogaça AC, Martins JR, Daffre S (2015) Low temperature affects cattle tick reproduction but does not lead to transovarial transmission of Anaplasma marginale. **Veterinary parasitology**, 214:322–326.

Farias NA da R (1995) Diagnóstico e controle da tristeza parasitária bovina.

Freebern E, Santos DJA, Fang L, Jiang J, Gaddis KLP, Liu GE, VanRaden PM, Maltecca C, Cole JB, Ma L (2020) GWAS and fine-mapping of livability and six disease traits in Holstein cattle. **BMC genomics**, 21:41.

Ganzinelli S, Rodriguez A, Schnittger L, Florin-Christensen M (2018) Babesia in Domestic Ruminants. In: **Parasitic Protozoa of Farm Animals and Pets**. [s.l.] Springer, p. 215–239.

Giglioti R, Oliveira HN, Santana CH, Ibelli AMG, Néo TA, Bilhassi TB, Rabelo MD, Machado RZ, Brito LG, Oliveira MC de S (2016) Babesia bovis and Babesia bigemina infection levels estimated by qPCR in Angus cattle from an endemic area of São Paulo state, Brazil. **Ticks and tick-borne diseases**, 7:657–662.

Giglioti R, Oliveira HN, Ibelli AMG, Bilhassi TB, Néo TA, Santana CH, Rabelo MD, Machado RZ, De Souza Chagas AC, De Sena Oliveira MC (2017) Neither quantification by qPCR nor quantitative Elisa can be used to discriminate Angus cattle for resistance/susceptibility to Babesia bovis. **Ticks and tick-borne diseases**, 8:335–340.

Goff WL, Johnson WC, Parish SM, Barrington GM, Tuo W, Valdez RA (2001) The age-related immunity in cattle to Babesia bovis infection involves the rapid induction of interleukin-12, interferon-γ and inducible nitric oxide synthase mRNA expression in the spleen. **Parasite immunology**, 23:463–471.

Gonçalves PM (2000) Epidemiologia e controle da tristeza parasitária bovina na região sudeste do Brasil. **Ciência Rural**, 30:187–194.

Hayes BJ, Goddard ME (2001) Prediction of total genetic value using genome-wide dense marker maps. **Genetics**, 157:1819–1829.

Hirsh DC, Burton GC, Blenden DC, Tsutakawa R (1974) The effect of tetracycline upon establishment of Escherichia coli of bovine origin in the enteric tract of man. **Journal of applied bacteriology**, 37:327–333.

Homer MJ, Aguilar-Delfin I, Telford SR, Krause PJ, Persing DH (2000) Babesiosis. **Clinical microbiology reviews**, 13:451–469.

Ibelli AMG, Ribeiro ARB, Giglioti R, Regitano LCA, Alencar MM, Chagas ACS, Paço AL, Oliveira HN de, Duarte JMS, Oliveira MCS (2012) Resistance of cattle of various genetic groups to the tick Rhipicephalus microplus and the relationship with coat traits. **Veterinary Parasitology**, 186:425–430.

Jonsson NN, Bock RE, Jorgensen WK (2008) Productivity and health effects of anaplasmosis and babesiosis on Bos indicus cattle and their crosses, and the effects of differing intensity of tick control in Australia. **Veterinary parasitology**, 155:1–9.

Kessler RH, Sacco AMS, Madruga CR, Muller M, Miguita M (1998) Teste crítico de vacinas atenuadas de Babesia bovis, B. bigemina e Anaplasma marginale em novilhas da raça Holandesa. **Revista Brasileira de Parasitologia Veterinária**, 7.

Kessler RH (2001) Considerações sobre a transmissão de Anaplasma marginale. **Pesquisa Veterinária Brasileira**, 21:177–179.

Kessler RH, Schenk MAM (1998) Carrapato, tristeza parasitária e tripanossomose dos bovinos. **Embrapa Gado de Corte-Livro técnico (INFOTECA-E)**.

Kocan KM, De La Fuente J, Blouin EF, Garcia-Garcia JC (2004) Anaplasma marginale (Rickettsiales: Anaplasmataceae): recent advances in defining host-pathogen adaptations of a tick-borne rickettsia. **Parasitology**, 129:S285.

Kocan KM, De La Fuente J, Blouin EF, Coetzee JF, Ewing SA (2010) The natural history of Anaplasma marginale. **Veterinary parasitology**, 167:95–107.

Ku CS, Loy EY, Pawitan Y, Chia KS (2010) The pursuit of genome-wide association studies: where are we now? **Journal of human genetics**, 55:195–206.

LEAL JJB (2009) Uso potencial de touros Braford em rebanho comercial. **Embrapa Pecuária Sul-Artigo de divulgação na mídia (INFOTECA-E)**.

Ma M, Chen Z, Liu A, Ren Q, Liu J, Liu Z, Li Y, Yin H, Guan G, Luo J (2016) Biological parameters of Rhipicephalus (Boophilus) microplus (Acari: Ixodidae) fed on rabbits, sheep, and cattle. **The Korean Journal of Parasitology**, 54:301.

Mahoney DF (1969) Bovine babesaisis: a study of factors concerned in transmission. **Annals of Tropical Medicine & Parasitology**, 63:1–14.

Mahoney DF, Ross DR (1972) Epizootiological factors in the control of bovine babesiosis. **Australian Veterinary Journal**, 48:292–298.

MAPA. (2020) **Agropecuária Brasileira em Números**. Disponível em: <https://www.gov.br/agricultura/pt-br/assuntos/politica-agricola/todas-publicacoes-de-politica-agricola/agropecuaria-brasileira-em-numeros>. Acesso em: 28 out. 2020.

Martins KR, Garcia MV, Bonatte-Junior P, Duarte PO, De Higa LOS, Csordas BG, Barros JC, Andreotti R (2020) Correlation between Rhipicephalus microplus ticks and Anaplasma marginale infection in various cattle breeds in Brazil. **Experimental and Applied Acarology**, 81:585–598.

Menezes A do NFRGR de O. (2016) **Papel do Zebu na pecuária de corte brasileira**. Disponível em: <https://www.embrapa.br/busca-de-noticias/-/noticia/9523901/artigo-papel-do-zebu-na-pecuaria-de-corte-brasileira>. Acesso em: 28 out. 2020.

Morand S, Beaudeau F, Cabaret J (2011) **New frontiers of molecular epidemiology of infectious diseases**. [s.l.] Springer Science & Business Media,

Moura AB, Vidotto O, Yamamura MH, Vidotto MC, Pereira AB da L (2003) Studies on the Anaplasma marginale Theiler, 1910 infection in Boophilus microplus (Canestrini, 1887) using 'nested'PCR. **Revista Brasileira de Parasitologia Veterinária**, 12:27–32.

Neto LRP, Jonsson NN, Michael JD, Barendse W (2011) Molecular genetic approaches for identifying the basis of variation in resistance to tick infestation in cattle. **Veterinary parasitology**, 180:165–172.

Obregón D, Corona-González B, Díaz-Sánchez AA, Armas Y, Roque E, De Sena Oliveira MC, Cabezas-Cruz A (2020) Efficient Transovarial Transmission of Babesia Spp. in Rhipicephalus microplus Ticks Fed on Water Buffalo (Bubalus bubalis). **Pathogens**, 9:280.

Ortega-Sánchez R, Camacho-Nuez M, Castañeda-Ortiz EJ, Martínez-Benítez MB, Hernández-Silva DJ, Aguilar-Tipacamú G, Mosqueda J (2020) Vaccine efficacy of recombinant BmVDAC on Rhipicephalus microplus fed on Babesia bigemina-infected and uninfected cattle. **Vaccine**,

Pereira TC (2018) **Introdução às técnicas de PCR convencional, em tempo real e digital**. [s.l: s.n.]

Piper EK, Jackson LA, Bagnall NH, Kongsuwan KK, Lew AE, Jonsson NN (2008) Gene expression in the skin of Bos taurus and Bos indicus cattle infested with the cattle tick, Rhipicephalus (Boophilus) microplus. **Veterinary immunology and immunopathology**, 126:110–119.

Ribeiro MF, Lima JD (1995) Influence of temperature on the development of Anaplasma marginale in Boophilus microplus. **Arq. bras. med. vet. zootec**, 525–533.

Ribeiro MFB, Lima JD (1996) Morphology and development of Anaplasma marginale in midgut of engorged female ticks of Boophilus microplus. **Veterinary parasitology**, 61:31–39.

Richardson IW, Berry DP, Wiencko HL, Higgins IM, More SJ, McClure J, Lynn DJ, Bradley DG (2016) A genome-wide association study for genetic susceptibility to Mycobacterium bovis infection in dairy cattle identifies a susceptibility QTL on chromosome 23. **Genetics Selection Evolution**, 48:19.

Riek RF (1964) The life cycle of Babesia bigemina (Smith and Kilborne, 1893) in the tick vector Boophilus microplus (Canestrini). **Australian Journal of Agricultural Research**, 15:802–821.

Ruiz PMG, Passos LMF, Ribeiro MFB (2005) Lack of infectivity of a Brazilian Anaplasma marginale isolate for Boophilus microplus ticks. **Veterinary parasitology**, 128:325–331.

Saleh MA (2009) Erythrocytic oxidative damage in crossbred cattle naturally infected with Babesia bigemina. **Research in Veterinary Science**, 86:43–48.

Sarli M, Novoa MB, Mazzucco MN, Signorini ML, Echaide IE, De Echaide ST, Primo ME (2020) A vaccine using Anaplasma marginale subdominant type IV secretion system recombinant proteins was not protective against a virulent challenge. **PloS one**, 15:e0229301.

Smith T, Kilborne FL (1893) **Investigations into the nature, causation, and prevention of Texas or southern cattle fever**. [s.l.] US Department of Agriculture, Bureau of Animal Industry,

Starcovici C (1893) Bemerkungen über den durch Babes entdeckten Blutparasiten und die durch denselben hervorgebrachten Krankheiten, die seuchenhafte Hämoglobinurie des Rindes (Babes), das Texasfieber (Th. Smith) und der Carceag der Schafe (Babes). **Zbl. Bakt., I. Abt**, 14:1–8.

Sutherst RW, Bourne AS (2006) The effect of desiccation and low temperature on the viability of eggs and emerging larvae of the tick, Rhipicephalus (Boophilus) microplus (Canestrini)(Ixodidae). **International Journal for Parasitology**, 36:193–200.

Tabor AE, Ali A, Rehman G, Rocha Garcia G, Zangirolamo AF, Malardo T, Jonsson NN (2017) Cattle tick Rhipicephalus microplus-host interface: a review of resistant and susceptible host responses. **Frontiers in Cellular and Infection Microbiology**, 7:506.

Theiler A. (1910) **Anaplasma marginale (gen. and spec. nov.): The marginal points in the blood of cattle suffering from a specific disease**. [s.l.] Pretoria: Government Printing and Stationary Office, .

Van Eenennaam AL, Weigel KA, Young AE, Cleveland MA, Dekkers JCM (2014) Applied animal genomics: results from the field. **Annu. Rev. Anim. Biosci.**, 2:105–139.

CHAPTER 2 - Genomic prediction and genome-wide Association Study for *Babesia bigemina* infection level in Braford and Hereford cattle

ABSTRACT - Babesiosis is a tick-borne disease that causes a negative economic impact on livestock farming in tropical countries. Information on the metabolic pathways involved in the immune response to bovine babesiosis caused by *Babesia bigemina* is still scarce in the literature. This study aimed to provide information about genetic aspects of BigIL by the estimation of heritability and genetic correlation between BigIL, *B. bov*is infection levels (BovIL), and tick count. Furthermore, genomic prediction and GWAS for BigIL were performed. To our knowledge, this is the first study to conduct genomics studies for BigIL. Blood samples were collected from 1,882 animals to measure BigIL. BigIL quantifications were obtained using real-time PCR method. Genotype information of this animals was imputed up to ~777,000 SNP. After the quality control, 1,880 animals and 502,398 SNPs remained for the subsequent analysis. The proportion of Nellore ancestry and heterozygosity was estimated in Braford breed to correct the phenotype. The variance components for BigIL, and genetic correlation between BigIL, tick count (n=5,867) and BovIL (n=1,858) were estimated using Bayesian approach. The single-step genomic best linear unbiased prediction method (GBLUP) was used to estimate breeding values and SNP effects. The heritability estimated for BigIL was low (0.090) which suggest a high environmental influence on this trait. A low genetic correlation was identified between BigIL and tick count (0.240), in contrast, BigIL and BovIL were highly correlated (0.624). This high correlation contributed to an improvement in the reliability of *B. bigemina* from 0.246 to 0.606. GWAS showed that BigIL trait is highly polygenic. The regions detected by GWAS analysis may be a promising region for future studies associated with BigIL, and contributed to the better understanding of the biological relationship between genetics and BigIL susceptibility in cattle

Keywords: Babesiosis; protozoa; enzootic stability.

2.1. Introduction

Babesiosis is a tick-borne disease caused by protozoa of the genus Babesia that has a great negative economic impact on livestock in tropical countries. Babesiosis has been characterized by high fever, lethargy, severe anemia, and hemoglobinuria, and can lead to death. Besides, *Babesia bigemina* is commonly found in conjunction with *Babesia bovis* and *Anaplasma marginale*, forming a parasitic complex called tick fever, causing an annual loss of around 18 billion dollars in the world (Neto et al., 2011; Bohaliga et al., 2018).

In areas of enzootic instability, there are specific periods of absence of tick *Rhipicephalus microplus* (main babesiosis vector) due to environmental factors, such as intense cold. Cattle born at this time are not exposed to the disease at a young age, favoring the emergence of outbreaks with animals presenting acute symptoms (BOCK et al., 1997). This is because young animals are protected by passive immunity from colostral antibodies and by non-specific factors (presence of thymus, fetal hemoglobin, etc) (Macedo, 2000).

Strategies commonly adopted to control the damages caused by tick-borne parasitic diseases include the use of acaricides and introgression of indicine breeds into taurine herds (Jonsson; Bock; Jorgensen, 2008). Genomic selection for tick resistance traits has shown promise for inclusion in animal breeding programs, with moderate and high accuracy results of 0.6 and 0.3 in Braford and Hereford cattle, respectively (Cardoso et al., 2015). However, the benefits of tick control in reducing infection rates and levels of *B. bigemina* are not still established. Cavani et al. (2020) found a low genetic correlation between *B. bovis* infection levels and tick count of 0.15, with 95% highest posterior density (HPD) interval varying from -0.147 to 0.445.

The improvement of genomic tools can contribute to the search for animals naturally resistant to *B. bigemina*. An example is the use of single nucleotide polymorphisms (SNP) in genome-wide association study (GWAS) to detect regions associated with the traits of interest. The SNPs can also be used in techniques for predicting the individual's genetic merit for later use in breeding programs (Van Eenennaam et al., 2014).

Although the economic relevance of bovine babesiosis mainly in tropical countries, to the best of our knowledge, there are no genomic studies for *B. bigemina* infection levels (BigIL). Thus, this study aimed to provide information about genetic aspects of BigIL by the estimation of heritability and genetic correlation between BigIL, *B. bov*is infection levels (BovIL), and tick count. Furthermore, we performed prediction ability and GWAS for BigIL.

2.2. Material and Methods

2.2.1. Animals, Management of Samples

To our knowledge, no quantitative genetic analysis has been performed for BigIL, and there are no reports in the literature of its correlation with BovIL and tick counts. Phenotypic data for BigIL were obtained from 1,882 animals of the Braford and Hereford breed raised in five farms located in the state of Rio Grande do Sul, Brazil, a region considered to be of enzootic instability. All the animals participate of the Delta G animal breeding program (Gensys Associated Consultants, RS, Brazil).

The individuals with BigIL and BovIL (n=1,858) phenotypes were distributed into contemporary groups (CG), constituted of management group and farm. Tick count (n=5,867) CG included farm, sex, year and season of birth (from 2008 to 2013), management group, and date of phenotypic evaluations. Linear and quadratic age in the tick count were considered as covariates for tick count.

To obtain the protozoan phenotype, DNA was extracted from the blood of animals using the Gensolve DNA recovery kit (Gentegra, Pleasanton, CA, USA). DNA quality and concentration were verified using NanoDrop spectrophotometer (NanoDrop Technologies Inc., Wilmington, Delaware, USA). The quantification of *B. bigemina* was based on the number of copies of the target sequence by quantitative polymerase chain reactions (qPCR) using CFX^{TM} Real-Time PCR Detection System (BioRad, Hercules, CA, USA).

The primers used were cbisg1 (forward) 5′-TGTTCCAGGAGATGTTGATTC-3′ and cbisg 2 (reverse) 5′-AGCATGGAAATAACGAAGTGC-3′ (Buling et al., 2007), flanking the cytochrome B of *B. bigemina* producing 88 base pair amplicons. Solutions containing known

concentrations of the *B. bigemina* copy number were analyzed together with the target DNA in the CFX96 system to estimate the number of copies in the samples. Samples and controls were tested in duplicates, when the number of copies was greater than zero and presented specific temperature melting (77.5 ± 0.5°C) they were considered positive for the infection. The same methodology was applied for *B. bovis*, as described by Cavani et al. (2020).

Tick count was obtained after weaning of animals naturally infested for *R. microplus.* The animals were submitted to visual inspection, when all bovines in the same contemporary group (animals raised together, receiving the same feeding and sanitary management) presented more than 20 engorged females, manual counting was carried. The number of ticks with more than 4.5 mm present on the right side of each animal was determined as tick count phenotype, as proposed by Hermans et al. (1994) and Utech et al. (1978). Tick counts were performed one to three times in each management group in late spring, summer, and early fall with at least 30 days between measures. All three phenotypes were transformed into log10 (n + 1) for an approximation of a normal distribution.

2.2.2. Genotype information

A total of 3,977 individuals were genotyped in panels of 27K (n=47), 30K (n=122), 50K (n=3,647), 150K (n=160), and HD (n=1). All the panels were imputed up to ~777,000 markers (Bovine HD) with software Beagle v. 5.1 (Browning; Zhou; Browning, 2018). The reference population was constituted by 115 Hereford, 113 Braford, and 1,184 Nelore breed. The SNP map used in the analysis was updated from UMD v3.1 assembly (Kinsella et al., 2011) to ARS-UCD1.2 coordinates (available from https://www.animalgenome.org/repository/cattle/UMC_bovine_coordinates/).

The accuracy of the imputation for each panel was assessed by the cross-validation method. The animals genotyped in HD were randomized and divided into five groups, each group was considered a validation group in a round (Figure 1). The genotype of the validation group was masked, and we kept just SNP present in the panel tested (27K, 30K, 50K, or 150K).

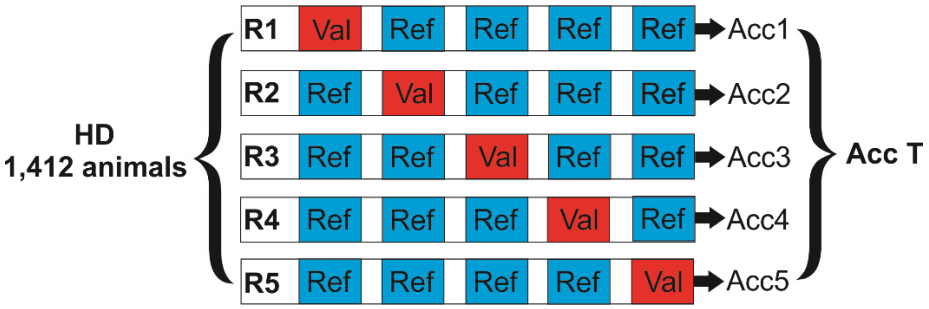

Figure 1. Cross-validation scheme, which the animals genotyped in HD were divided into five groups and each of them was a validation group (Val) once, totalizing five rounds (R1, R2, R3, R4, and R5) for each panel tested (27K, 30K, 50K, and 150K). The genotype of the Val group was reduced for the same number of the SNP of the panel tested. The accuracy was calculated for each round, and the mean of the five values (Acc1, Acc2, Acc3, Acc4, and Acc5) generated composed the imputation accuracy (Acc T).

The accuracy of imputation was calculated as the concordance rate (CR), which considered the proportion of genotype correctly imputed, and the individual accuracy was obtained by Pearson correlation (r) between imputed genotype and true genotype (Table 1).

Table 1. Accuracy of imputations measures for SNP by concordance rate (CR) and by animal using Pearson correlation (r), and their respective standard deviation.

Panel	CR	r
27K	0.979 ± 0.021	0.971 ± 0.019
30K	0.977 ± 0.022	0.978 ± 0.015
50K	0.972 ± 0.020	0.952 ± 0.05
150K	0.989 ± 0.010	0.987 ± 0.011

Genotype data were submitted to quality control (QC) before and after imputation. The parameters of exclusion used for QC before imputation were: SNP with a call rate ≤ 0.95, minor allele frequency (MAF) ≤ 2%, P-value in the Hardy-Weinberg equilibrium test < 10-5, non-autosomal markers, and different SNPs mapped to the same genomic coordinate. The exclusion criteria for samples were call rate ≤ of 0.90 and minimum CG size of four animals. QC procedures after imputation were the same described above but without the Hardy-Weinberg equilibrium parameter. QC of markers and samples were performed through Plink v.1.9 (Chang et al., 2015). After QC, 502,398 SNPs

remained for the next analysis. The samples remained for TC, BigIL, and BovIL were 5,867, 1,880, 1,858, respectively.

2.2.3. Estimates of Nelore proportion and Heterozygosity in Braford Breed

Brazilian Braford (Nelore x Hereford) has a variation in breed composition which can have influence in cattle resistance associated with indicine breed proportion (considered more resistant to diseases than taurine). So LAMP-ANC v. 2.5 (Sankararaman et al., 2008) was used to estimate genomic heterozygosity and Nelore proportion in the crossbreed. It was assumed the ancestral population (K) equals 2, corresponding to Hereford and Nelore breeds.

For both analyses, it was considered known ancestry, using genotypic information from 2,376 animals (1,184 Nelore and 1,192 Hereford) as reference individuals. The output obtained from LAMP-ANC coded a single locus as 0, 0.5, or 1 where 0.5 represents the heterozygous genotype. When both alleles came from different ancestries (0.5 in each allele corresponding to the same locus) were computed as 1, and alleles that came from the same ancestral was 0, the sum of these results was divided by the total of SNPs; following the methodology applied by Khayatzadeh et al. (2019).

According to preliminary analysis using *lm* function in R software v. 4.0.3 (Team, 2013), heterozygosity explained most of the variance of the phenotypes rather than Nelore proportion, and given the high correlation among them, only heterozygosity was used to correct the phenotypes.

2.2.4. Variance components estimation

A multi-trait animal model under Bayesian inference were used to estimate variance components for BigIL, and their correlation with BovIL and tick count. The model can be represented as follows:

$$y = Xb + Zu + Pp + e$$

where $\boldsymbol{y}$ is the vector of the phenotypic records, $\boldsymbol{b}$ is the vector of fixed effects (CG), and the individual heterozygosity as co-variable. The additive genetic value for individuals was $\boldsymbol{u}|\mathbf{G} \sim N(0, \mathbf{G} \otimes \sigma_u)$, the permanent environment was defined as $\boldsymbol{p}|P \sim N(0, \mathbf{I} \otimes \sigma_p)$ and the residuals as $\boldsymbol{e} \sim N(0, \mathbf{I} \otimes \sigma_e)$. The $\boldsymbol{X}$, $\boldsymbol{Z}$ and $\boldsymbol{P}$ are the incidence matrices. **G** is the genomic relationship matrix created by PREGSF90

software based on VanRaden (2008) method calculated as $\boldsymbol{G}=ZZ'/[2\sum_{j=1}^{m}P_j(1-P)]$, where Z is the SNP matrix of markers. Permanent environment effect were included in the model only for tick count phenotype. The statistical analyses were performed using GIBBS2F90 and POSTGIBBSF90 from the BLUPF90 family software (Misztal et al., 2002), and consisted of 500,000 cycles with burn-in of 50,000, taking sample each every 10 iterations resulting in 44,999 to obtain the parameters. Chain convergence were tested by visual examination in R software.

2.2.5. Genomic Prediction and Genome-wide Association Study

As suggested by Engle et al. (2018) for the cross-validation analysis, the animals were randomized and divided into 5 groups of 376 animals (20% of total) using each division as a validation group (phenotype assumed as unknown) and the other four groups as a reference, this procedure was performed 10 times. Prediction accuracies were calculated as the Pearson correlation between adjusted phenotype and GEBVs estimated, divided by the square root of the estimated heritability. For the prediction of the genomic breeding values, we used BLUPF90 software, applying a single, and multivariate model.

The SNP effects were obtained from the GEBVs of the animals using postGSf90 software, which calculates a weight for each SNP. Therefore, PreGSf90 followed of BLUPF90 and postGSf90 were carried twice with different weights in G matrix as described by Zhang et al. (2016).

To search for evidence that complements GWAS results about the association between SNPs with higher effects and BigIL, regions within 500 kb on either side of the most favorable SNP were analyzed. BioMart tool in the Ensembl Genes 94 was used to obtain gene coordinates in the UMD v3.1 assembly (Kinsella et al., 2011). The CattleQTLdb v.42 (Hu et al., 2013) was examined to check if any associated genomic region was previously reported as a cattle quantitative trait locus (QTL). Gene Ontology (GO) enrichment analysis using Database for Annotation, Visualization, and Integrated Discovery (DAVID) (Sherman; Lempicki, 2009) was consulted to identify genes functions, and a review of the literature around these genes was performed.

2.3. Results and Discussion

The ancestry proportion of Braford breed estimated showed an average of 32% of Nelore and 68% of Hereford, with a heterozygosity range of 0.46 (±0.09). In Braford breed, BigIL was identified with the minimum, average, and maximum equal 0.00, 1.48, and 4.08, respectively, with 437 non-infected animals. BigIL in Hereford was quantified as a minimum, average, and a maximum of 0.00, 4.12, 2.12, 2.22, respectively with 17 non-infected animals. Similar results were observed by Bilhassi et al. (2014) and Oliveira et al. (2008) that reported higher resistance (lower parasitemia) to *B. bigemina* and *B. bovis* in indicine and its crosses breed than in pure taurine. Bock et al. (1997) and Jonsson et al. (2008) also highlighted differences in immune responses of *B. indicus* against babesiosis. Regarding the influence of the crossbreed on the variation of parasitemia rates in the present population, the increase of heterozygosity represented a decrease of 0.88 in the copy number of *B. bigemina* ($P<0.01$). Cardoso et al. (2015) identified a favorable heterozygosity effect on tick resistance.

Genetic parameters for BigIL are summarized in Table 2. The estimates were low for additive genetic variance (0.069) and heritability (0.090). These results express the high environmental influence on the BigIL trait.

Table 2. Posterior means, with 95% interval of highest posterior density (HPD) in parentheses of the additive genetic variance (σ_u), heritability (h^2), and genetic correlation estimates (r).

σ_u	0.069 (0.013; 0.125)
h^2	0.090 (0.017; 0.161)
$R_{BigILxTC}$	0.240 (-0.05; 0.546)
$R_{BigILxBovIL}$	0.624 (-0.310; 0.999)

*TC = tick count trait; BigIL = *B. bigemina* infection level; BovIL = *B. bovis* infection level.

A similar value of h^2 was estimated for BovIL in the same population using Bayesian analysis (0.07 with variation from 0.037 to 0.124) as reported by (Cavani et al., 2020). Disease resistance traits in bovine have also presented low heritability such as those caused by bacterial pathogens in cows (from 0.02±0.01 to 0.13±0.02) and fungal pathogen (0.04±0.01) (Mahmoud et al., 2018). However, Berry et al. (2011) suggested that resistance to most bovine diseases does not

have a purely environmental progression, with genetic variation in resistance, even with the low heritability.

Our result of the genetic correlation estimated was low between tick count and BigIL (0.24), and we did not find in the literature estimates of genetic correlation between BigIL and tick count. However, Maiorano et al. (2019) and Cavani et al. (2020) also reported a low phenotypic (-0.07) and genetic (0.15) correlation between tick count and *B. bovis*. The low correlation indicates that genetic factors influencing tick quantity are poorly related to parasitemia. This hypothesis is corroborated by Oliveira-Sequeira et al. (2005), who analyzed tick infection by *B. bigemina*, and found that an infected host may contain non-protozoan-contaminated ticks. There are also reports showing no correlation between infected ticks and the presence of protozoa in the bovine host (Hector et al., 2019), and a unique infected tick has been described as sufficient to transmit babesiosis (Bock et al., 2004).

In contrast to the result between BigIL and tick count, the genetic correlation between BigIL and BovIL was high (0.624) suggesting that breeding using resistance to *B. bigemina* would also have effects on the levels of infection by *B. bovis.* An experiment performed by Wright et al. (1987) indicated that animals previously infected with *B. bigemina*, when challenged by *B. bovis* have not developed an acute phase of the disease, suggesting that common immune defense mechanisms are acting in both traits.

The accuracy prediction estimates using a univariate model for BigIL were low (0.246±0.189). The values of the ability of prediction using a univariate model indicate that incorporation of more phenotypic information is needed to improve the genomic prediction accuracy (Dehnavi et al. 2018). The authors achieved gains in accuracy of 0.16 with the increase of phenotypic information of bulls and cows in the training population for a trait with 0.05 of heritability.

In a multivariate model, the ability of prediction increased to 0.606±0.180. As suggested by Karoui et al. (2012) correlated traits can improve the accuracy of the breeding value, in this sense, the correlation between BigIL and BovIL may have contributed to improving genomic prediction accuracy gains using the multivariate model. The gain in accuracy in a multivariate model was also observed by Michenet et al. (2019) and Jenko et al. (2017) which identified an increase of 0.07 and 0.16, respectively, in comparison with the univariate model.

According to Thompson and Meyer, (1986) gains in accuracy can be greater when one of the traits has high heritability. The multi-trait model can still be explored as information on traits correlated with BigIL is reported with higher heritability,

GWAS result indicated that the BigIL trait has a polygenic inheritance, which is determined by a large number of genetic variants with small effects (Fig. 2).

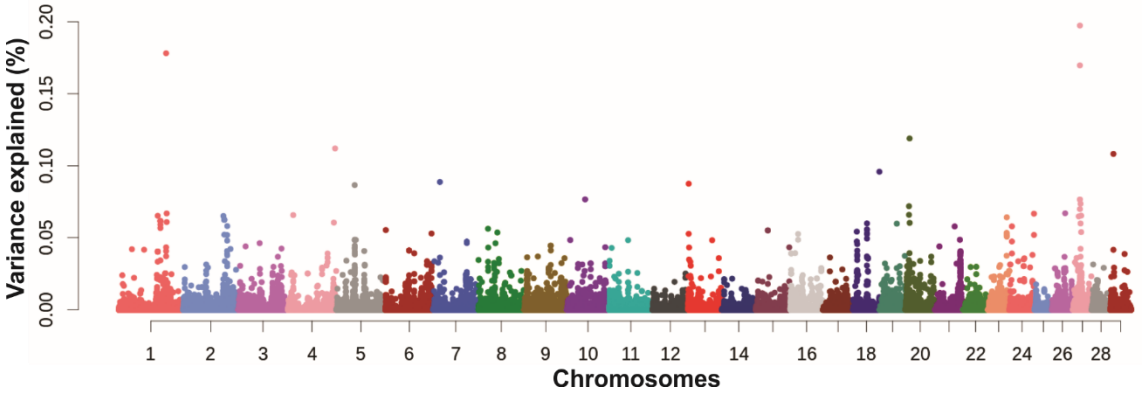

Figure 2. Manhattan plot of the additive genetic variance explained by each SNP for *B. bigemina* infection level.

The regions of the top 10 SNP overlapped with a total of 104 genes (Supplementary Table 1) and 190 QTL (Supplementary Table 2). In the regions explored were identified QTL previously related to health, milk, growth, and reproduction, indicating that QTL related with resistance to BigIL can be evolved in other economically important traits. Enrichment analysis revealed the involvement of 17 genes related to host defense (Table 3). These genes play role in the biological process of Bacteriolytic enzyme (GO:0008152), defense response to Gram-positive bacterium (GO:0050830), defense response to Gram-negative bacterium (GO:0050829), Antimicrobial (GO:0019730), and Disulfide bond (GO:0015035).

Table 3. Genes positioned into a windows around of the top ten regions indicated by GWAS related with immune response based on DAVID enrichment.

Gene ID	Chr	Gene (start-end)	Gene Name	Description
ENSBTAG00000004826	7	15911386-15915021	*ACP5*	acid phosphatase 5

ENSBTAG00000047486	20	9865315-9867404	*CARTPT*	CART prepropeptide
ENSBTAG00000007312	7	16586871-16589914	*CD209*	CD209 molecule
ENSBTAG00000013429	7	16561209-16564690	*CLEC4G*	C-type lectin domain family 4 member G
ENSBTAG00000008197	7	15775449-15781166	*EPOR*	erythropoietin receptor
ENSBTAG00000020657	27	16420193-16546078	*FAT1*	FAT atypical cadherin 1
ENSBTAG00000012687	7	16058828-16183062	*INSR*	insulin receptor
ENSBTAG00000026323	5	44347280-44352877	*LYSB*	intestinal lysozyme
ENSBTAG00000026779	5	44506989-44515796	*LYZ*	lysozyme
ENSBTAG00000046511	5	44392339-44400094	*LYZ1*	lysozyme (renal amyloidosis)
ENSBTAG00000026088	5	44365581-44371561	*LYZ2*	lysozyme C-2
ENSBTAG00000046628	5	44423778-44430774	*LYZ3*	lysozyme 3
ENSBTAG00000046938	27	16383422-16406473	*MTNR1A*	melatonin receptor 1A
ENSBTAG00000021945	10	44846568-44939145	*NID2*	nidogen 2
ENSBTAG00000020990	1	116847497-116879544	*P2RY14*	purinergic receptor P2Y14
ENSBTAG00000004716	7	16513147-16514562	*RETN*	resistin
ENSBTAG00000011225	10	44057645-44070733	*TMX1*	thioredoxin related transmembrane protein 1

In addition to the genes identified by DAVID, in Table 4 are listed genes previously reported as associated with immunity based on a review of the literature.

Table 4. Genes positioned into a windows around of the top ten regions indicated by GWAS related with immune response based on review of the literature.

Gene ID	Chr	Gene (start-end)	Gene Name	Description
ENSBTAG00000001728	1	116623017-116652023	*IGSF10*	immunoglobulin superfamily member 10
ENSBTAG00000004270	7	16531763-16544273	*FCER2*	Fc fragment of IgE receptor II
ENSBTAG00000000561	20	10230183-10276416	*OCLN*	occludin
ENSBTAG00000002073	20	10341517-10375453	*RAD17*	RAD17 checkpoint

				clamp loader component
ENSBTAG00000000363	20	10017703-10086104	*BDP1*	"B double prime 1
ENSBTAG00000007312	7	16586871-16589914	*CD209*	CD209 molecule
ENSBTAG00000008197	7	15775449-15781166	*EPOR*	erythropoietin receptor
ENSBTAG00000011207	7	15883405-15891295	*CNN1*	calponin 1
ENSBTAG00000008967	5	45157005-45200873	*RAP1B*	RAP1B"
ENSBTAG00000010422	5	44979892-45005325	*MDM2*	MDM2 proto-oncogene
ENSBTAG00000046511	5	44392339-44400094	*LYZ1*	lysozyme (renal amyloidosis)
ENSBTAG00000026088	5	44365581-44371561	*LYZ2*	lysozyme C-2
ENSBTAG00000046628	5	44423778-44430774	*LYZ3*	lysozyme 3

The *IGSF10* belongs to the immunoglobulin (Ig) superfamily, which is involved with immune response by the antigen presentation (Cruz-Tapias; Castiblanco; Anaya, 2013). The *FCER2* also expresses an antigen-presenting cell mediate by the IgE (Chan). *OCLN,* and *RAD17* genes have previously been associated with mammary immune response in humans and cattle (Werner-Misof et al., 2007; Kirsanova et al., 2020). The *BDP1* expressed in the placenta of cows was related to Brucella inflammatory response (Mol et al., 2014).

Kumar et al. (2020) and Vázquez et al. (2014) reported the association between *CD209* gene and variability of resistance to paratuberculosis in bovine. The genes *EPOR*, *CNN1*, and *RAP1B* were reported to act in a cytokine-cytokine receptor interaction pathway related to *Staphylococcus aureus* infection resistance, which can cause mastitis (Fang et al., 2016; Wang et al., 2020, 2021). The gene *MDM2* was previously related to susceptibility to the bovine infection by the tick-disease caused by the protozoan parasite of the genus Theileria (Hayashida et al., 2013). The genes *LIZ1*, *LIZ2*, and *LIZ3* were reported as upregulated in resistant cattle to intestinal worms infection, rather than susceptible animals (Li et al., 2015). These genes are also related to lysozyme expression which is an important substance present in the colostrum, acting in the passive immunity of calves (Puppel et al., 2020).

Gene location identified from GWAS analyses was also associated with adaptation, such as *PLCB1*, *PAXIP1,* and *P2RY14* related with oxidative stress and adaptation to hot environments in beef and dairy cattle (Seo et al., 2014; Li et al., 2015; Casas; Ma; Lippolis, 2020). *GPR171* was associated with altitude

adaptation (Verma et al., 2018), and *RAB38* coat color and ocular hypopigmentation in Holstein (Sherman; Lempicki, 2009)

2.4. Conclusion

Despite the small magnitude of the heritabilities for BigIL, our results indicate the possibility of improving this trait. The use of the multivariate model allowed a better understanding of the genetic correlation between BigIL and BovIL and contributed to the increasing ability of accuracy for BigIL, showing that BovIL should be included as breeding objectives for BigIL. On the other hand, the inclusion of the tick count measures does not improve the prediction ability for BigIL. The regions detected by GWAS analysis may be a promising region for future studies associated with BigIL and contributed to the better understanding of the biological relationship between genetic factors and resistance to infection with *B. bigemina* in cattle.

2.5. References

Berry DP, Bermingham ML, Good M, More SJ (2011) Genetics of animal health and disease in cattle. **Irish Veterinary Journal**, 64:5.

Bilhassi TB, Oliveira HN, Ibelli AMG, Giglioti R, Regitano LCA, Oliveira-Sequeira TCG, Bressani FA, Malagó Jr W, Resende FD, Oliveira MCS (2014) Quantitative study of Babesia bovis infection in beef cattle from São Paulo state, Brazil. **Ticks and tick-borne diseases**, 5:234–238.

Bock R, Jackson L, De Vos A, Jorgensen W (2004) Babesiosis of cattle. **Parasitology**, 129:S247–S269.

Bock RE, De Vos AJ, Kingston TG, McLellan DJ (1997) Effect of breed of cattle on innate resistance to infection with Babesia bovis, B bigemina and Anaplasma marginale. **Australian veterinary journal**, 75:337–340.

Browning BL, Zhou Y, Browning SR (2018) A one-penny imputed genome from next-generation reference panels. **The American Journal of Human Genetics**, 103:338–348.

Buling A, Criado-Fornelio A, Asenzo G, Benitez D, Barba-Carretero JC, Florin-Christensen M (2007) A quantitative PCR assay for the detection and quantification of Babesia bovis and B. bigemina. **Veterinary parasitology**, 147:16–25.

Cardoso FF, Gomes CCG, Sollero BP, Oliveira MM, Roso VM, Piccoli ML, Higa RH, Yokoo MJ, Caetano AR, Aguilar I (2015) Genomic prediction for tick

resistance in Braford and Hereford cattle. **Journal of Animal Science**, 93:2693–2705.

Casas E, Ma H, Lippolis JD (2020) Expression of viral microRNAs in serum and white blood cells of cows exposed to bovine leukemia virus. **Frontiers in Veterinary Science**, 7.

Cavani L, Braz CU, Giglioti R, Okino CH, Gulias-Gomes CC, Caetano AR, De Sena Oliveira MC, Cardoso FF, De Oliveira HN (2020) Genomic study of Babesia bovis infection level and its association with tick count in Hereford and Braford cattle. **Frontiers in Immunology**, 11.

Chang CC, Chow CC, Tellier LCAM, Vattikuti S, Purcell SM, Lee JJ (2015) Second-generation PLINK: rising to the challenge of larger and richer datasets. **Gigascience**, 4:7.

Cruz-Tapias P, Castiblanco J, Anaya J-M (2013) Major histocompatibility complex: antigen processing and presentation. In: **Autoimmunity: From Bench to Bedside.**

Dehnavi E, Mahyari SA, Schenkel FS, Sargolzaei M (2018) The effect of using cow genomic information on accuracy and bias of genomic breeding values in a simulated Holstein dairy cattle population. **Journal of dairy science**, 101:5166–5176.

Fang L, Hou Y, An J, Li B, Song M, Wang X, Sørensen P, Dong Y, Liu C, Wang Y (2016) Genome-wide transcriptional and post-transcriptional regulation of innate immune and defense responses of bovine mammary gland to Staphylococcus aureus. **Frontiers in cellular and infection microbiology**, 6:193.

Giglioti R, Oliveira HN, Santana CH, Ibelli AMG, Néo TA, Bilhassi TB, Rabelo MD, Machado RZ, Brito LG, Oliveira MC de S (2016) Babesia bovis and Babesia bigemina infection levels estimated by qPCR in Angus cattle from an endemic area of São Paulo state, Brazil. **Ticks and tick-borne diseases**, 7:657–662.

Hayashida K, Kajino K, Hattori M, Wallace M, Morrison I, Greene MI, Sugimoto C (2013) MDM2 regulates a novel form of incomplete neoplastic transformation of Theileria parva infected lymphocytes. **Experimental and molecular pathology**, 94:228–238.

Hector E, Elelu N, Ferrolho J, Couto J, Sanches G, Antunes S, Domingos A, Eisler M (2019) PCR detection of Ehrlichia ruminantium and Babesia bigemina in cattle from Kwara State, Nigeria: unexpected absence of infection. **Parasitology Research**, 118:1025–1029. Disponível em: <https://doi.org/10.1007/s00436-019-06204-1>.

Hermans D, Houwer J De, Eelen P (1994) The affective priming effect: Automatic activation of evaluative information in memory. **Cognition & Emotion**, 8:515–533.

Jenko J, Wiggans GR, Cooper TA, Eaglen SAE, Luff WG de L, Bichard M, Pong-

Wong R, Woolliams JA (2017) Cow genotyping strategies for genomic selection in a small dairy cattle population. **Journal of dairy science**, 100:439–452.

Jonsson NN, Bock RE, Jorgensen WK (2008) Productivity and health effects of anaplasmosis and babesiosis on Bos indicus cattle and their crosses, and the effects of differing intensity of tick control in Australia. **Veterinary parasitology**, 155:1–9.

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

Khayatzadeh N, Mészáros G, Utsunomiya YT, Schmitz-Hsu F, Seefried F, Schnyder U, Ferenčaković M, Garcia JF, Curik I, Sölkner J (2019) Genome-wide mapping of the dominance effects based on breed ancestry for semen traits in admixed Swiss Fleckvieh bulls. **Journal of dairy science**, 102:11217–11224.

Kirsanova E, Heringstad B, Lewandowska-Sabat A, Olsaker I (2020) Identification of candidate genes affecting chronic subclinical mastitis in Norwegian Red cattle: combining genome-wide association study, topologically associated domains and pathway enrichment analysis. **Animal Genetics**, 51:22–31.

Kumar S, Kumar S, Singh RV, Chauhan A, Kumar A, Bharati J, Singh SV (2020) Association of genetic variability in CD209 gene with bovine paratuberculosis disease: a case–control study in the Indian cattle population. **Animal Biotechnology**, 1–8.

Li RW, Wu S, Li C-J, Li W, Schroeder SG (2015) Splice variants and regulatory networks associated with host resistance to the intestinal worm Cooperia oncophora in cattle. **Veterinary parasitology**, 211:241–250.

Mahmoud M, Zeng Y, Shirali M, Yin T, Brügemann K, König S, Haley C (2018) Genome-wide pleiotropy and shared biological pathways for resistance to bovine pathogens. **Plos one**, 13:e0194374.

Maiorano AM, Giglioti R, Oliveira MCS, Oliveira HN, Cyrillo J, Mercadante MEZ, Silva J (2019) Resistance to the tick Rhipicephalus microplus and Babesia bovis infection levels in beef heifers raised in an endemic area of Sao Paulo state, Brazil. **Animal Production Science**, 59:938–944.

Michenet A, Boichon D, Saintilan R, Phocas F, Venot E (2019) A single-step, multiple-trait genomic evaluation model increase the accuracy for suckling performance in beef cows. **ICAR Technical Series**, 33–39.

Misztal I, Tsuruta S, Strabel T, Auvray B, Druet T, Lee DH (2002) BLUPF90 and related programs. Communication no. 28-07. In: Proc. 7th World Congress on Genetics Applied to Livestock Production, Montpellier, France, **Anais**.

Mol JPS, Costa EA, Carvalho AF, Sun Y-H, Tsolis RM, Paixão TA, Santos RL (2014) Early Transcriptional Responses of Bovine Chorioallantoic Membrane Explants to Wild Type, Δ virB2 or Δ btpB Brucella abortus Infection. **PloS one**,

9:e108606.

Oliveira-Sequeira TCG, Oliveira MC de S, Araujo Jr JP, Amarante AFT (2005) PCR-based detection of Babesia bovis and Babesia bigemina in their natural host Boophilus microplus and cattle. **International journal for parasitology**, 35:105–111.

Oliveira MCS, Oliveira-Sequeira TCG, Regitano LCA, Alencar MM, Néo TA, Silva AM, Oliveira HN de (2008) Detection of Babesia bigemina in cattle of different genetic groups and in Rhipicephalus (Boophilus) microplus tick. **Veterinary parasitology**, 155:281–286.

Puppel K, Gołębiewski M, Grodkowski G, Solarczyk P, Kostusiak P, Klopčič M, Sakowski T (2020) Use of somatic cell count as an indicator of colostrum quality. **Plos one**, 15:e0237615.

Sankararaman S, Sridhar S, Kimmel G, Halperin E (2008) Estimating local ancestry in admixed populations. **The American Journal of Human Genetics**, 82:290–303.

Seo J, Osorio JS, Schmitt E, Corrêa MN, Bertoni G, Trevisi E, Loor JJ (2014) Hepatic purinergic signaling gene network expression and its relationship with inflammation and oxidative stress biomarkers in blood from peripartal dairy cattle. **Journal of dairy science**, 97:861–873.

Sherman BT, Lempicki RA (2009) Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. **Nature protocols**, 4:44.

Team RC (2013) R: A language and environment for statistical computing.

Thompson R, Meyer K (1986) A review of theoretical aspects in the estimation of breeding values for multi-trait selection. **Livestock Production Science**, 15:299–313.

Utech KBW, Wharton RH, Kerr JD (1978) Resistance to Boophilus microplus (Canestrini) in different breeds of cattle. **Australian Journal of Agricultural Research**, 29:885–895.

Vázquez P, Ruiz-Larrañaga O, Garrido JM, Iriondo M, Manzano C, Agirre M, Estonba A, Juste RA (2014) Genetic association analysis of paratuberculosis forms in Holstein-Friesian cattle. **Veterinary medicine international**, 2014.

Verma P, Sharma A, Sodhi M, Thakur K, Kataria RS, Niranjan SK, Bharti VK, Kumar P, Giri A, Kalia S (2018) Transcriptome analysis of circulating PBMCs to understand mechanism of high altitude adaptation in native cattle of ladakh region. **Scientific reports**, 8:1–15.

Wang D, Liu L, Augustino SMA, Duan T, Hall TJ, MacHugh DE, Dou J, Zhang Y, Wang Y, Yu Y (2020) Identification of novel molecular markers of mastitis caused by Staphylococcus aureus using gene expression profiling in two consecutive generations of Chinese Holstein dairy cattle. **Journal of animal science and biotechnology**, 11:1–17.

Wang X, Fan Y, Han Z, Gong Z, Peng Y, Mao Y, Yang Z, Yang Y (2021) Integrative Analysis of miRNA and mRNA Expression Profiles in Mammary Glands of Holstein Cows Artificially Infected with Staphylococcus Aureus.

Werner-Misof C, Pfaffl MW, Meyer HHD, Bruckmaier RM (2007) Effect of chronic oxytocin-treatment on the bovine mammary gland immune system. **Veterinarni Medicina-Praha-**, 52:475.

Wright IG, Goodger B V, Leatch G, Aylward JH, Rode-Bramanis K, Waltisbuhl DJ (1987) Protection of Babesia bigemina-immune animals against subsequent challenge with virulent Babesia bovis. **Infection and immunity**, 55:364–368.

Zhang X, Lourenco D, Aguilar I, Legarra A, Misztal I (2016) Weighting strategies for single-step genomic BLUP: an iterative approach for accurate calculation of GEBV and GWAS. **Frontiers in genetics**, 7:151.

2.6. Supplementary material

Supplementary table 1. Genes positioned into a Windows of 1Mb to the top ten regions indicated by GWAS.

Gene ID	Chr	Windows (start-end)	Gene (start-end)	Gene Name	Description
ENSBTAG00000000363	20	9387590-10387590	10017703-10086104	BDP1	"B double prime 1
ENSBTAG00000000561	20	9387590-10387590	10230183-10276416	OCLN	occludin
ENSBTAG00000000613	7	15770431-16770431	16714677-16752222	ELAVL1	ELAV like RNA binding protein 1
ENSBTAG00000001728	1	116417195-117417195	116623017-116652023	IGSF10	immunoglobulin superfamily member 10
ENSBTAG00000001961	20	9387590-10387590	9401263-9494370	MAP1B	microtubule associated protein 1B
ENSBTAG00000001962	20	9387590-10387590	9285735-9390606	MRPS27	mitochondrial ribosomal protein S27
ENSBTAG00000002073	20	9387590-10387590	10341517-10375453	RAD17	RAD17 checkpoint clamp loader component
ENSBTAG00000002629	7	15770431-16770431	16368804-16373988	TEX45	testis expressed 45
ENSBTAG00000003043	10	43913775-44913775	44659979-44794168	GNG2	G protein subunit gamma 2
ENSBTAG00000003261	18	64431617-65431617	64453980-64458059	AURKC	aurora kinase C
ENSBTAG00000003326	20	9387590-10387590	10147256-10176131	NAIP	NLR family apoptosis inhibitory protein
ENSBTAG00000004268	7	15770431-16770431	16526036-16528271	TRAPPC5	trafficking protein particle complex 5
ENSBTAG00000004270	7	15770431-16770431	16531763-16544273	FCER2	Fc fragment of IgE receptor II
ENSBTAG00000004294	7	15770431-16770431	16608165-16640661	EVI5L	ecotropic viral integration site 5 like
ENSBTAG00000004300	7	15770431-16770431	16653091-16660988	LRRC8E	leucine rich repeat containing 8 VRAC subunit E
ENSBTAG00000004714	7	15770431-16770431	16521617-16525532	MCEMP1	mast cell expressed membrane protein 1
ENSBTAG00000004716	7	15770431-16770431	16513147-16514562	RETN	resistin
ENSBTAG00000004826	7	15770431-16770431	15911386-15915021	ACP5	acid phosphatase 5"

ENSBTAG00000005592	7	15770431-16770431	16390698-16399664	MCOLN1	mucolipin 1
ENSBTAG00000005743	20	9387590-10387590	10105978-10137931	SMN2	"survival of motor neuron 2
ENSBTAG00000006911	5	44288183-45288183	45077381-45125506	NUP107	nucleoporin 107
ENSBTAG00000007312	7	15770431-16770431	16586871-16589914	CD209	CD209 molecule
ENSBTAG00000007323	5	44288183-45288183	44560547-44588478	CPSF6	cleavage and polyadenylation specific factor 6
ENSBTAG00000008196	7	15770431-16770431	15772589-15774930	SWSAP1	SWIM-type zinc finger 7 associated protein 1
ENSBTAG00000008197	7	15770431-16770431	15775449-15781166	EPOR	erythropoietin receptor
ENSBTAG00000008198	7	15770431-16770431	15785486-15800362	RGL3	ral guanine nucleotide dissociation stimulator like 3
ENSBTAG00000008201	7	15770431-16770431	15800804-15811483	CCDC151	coiled-coil domain containing 151
ENSBTAG00000008202	7	15770431-16770431	15811857-15825182	PRKCSH	protein kinase C substrate 80K-H
ENSBTAG00000008338	13	937036-1937036	933264-1794219	PLCB1	phospholipase C beta 1
ENSBTAG00000008906	7	15770431-16770431	16476406-16477872	PCP2	Purkinje cell protein 2
ENSBTAG00000008967	5	44288183-45288183	45157005-45200873	RAP1B	RAP1B"
ENSBTAG00000009178	7	15770431-16770431	16482854-16491641	STXBP2	syntaxin binding protein 2
ENSBTAG00000009460	18	64431617-65431617	64754096-64764864	ZNF550	zinc finger protein 550
ENSBTAG00000009848	1	116417195-117417195	116891760-116898285	GPR171	G protein-coupled receptor 171
ENSBTAG00000010103	10	43913775-44913775	43826973-43944784	TRIM9	tripartite motif containing 9
ENSBTAG00000010422	5	44288183-45288183	44979892-45005325	MDM2	MDM2 proto-oncogene
ENSBTAG00000010639	7	15770431-16770431	16662127-16672289	MAP2K7	mitogen-activated protein kinase kinase 7
ENSBTAG00000011100	29	7127813-8127813	7375472-7417234	CTSC	cathepsin C
ENSBTAG00000011192	1	116417195-117417195	116756155-116798069	GPR87	G protein-coupled receptor 87
ENSBTAG00000011207	7	15770431-16770431	15883405-15891295	CNN1	calponin 1
ENSBTAG00000011225	10	43913775-44913775	44057645-44070733	TMX1	thioredoxin related transmembrane protein 1

ENSBTAG00000011267	1	116417195-117417195	117161151-117204819	CLRN1	clarin 1
ENSBTAG00000011356	7	15770431-16770431	16447830-16464459	CAMSAP3	calmodulin regulated spectrin associated protein family member 3
ENSBTAG00000011360	7	15770431-16770431	16450200-16474545	XAB2	XPA binding protein 2
ENSBTAG00000012687	7	15770431-16770431	16058828-16183062	INSR	insulin receptor
ENSBTAG00000013429	7	15770431-16770431	16561209-16564690	CLEC4G	C-type lectin domain family 4 member G
ENSBTAG00000013496	5	44288183-45288183	44876588-44960458	CPM	carboxypeptidase M
ENSBTAG00000014412	7	15770431-16770431	15828841-15844379	ELAVL3	ELAV like RNA binding protein 3
ENSBTAG00000014415	7	15770431-16770431	15846201-15863557	ZNF653	zinc finger protein 653
ENSBTAG00000014926	7	15770431-16770431	16674793-16679420	SNAPC2	small nuclear RNA activating complex polypeptide 2
ENSBTAG00000015049	7	15770431-16770431	15862527-15874429	ECSIT	ECSIT signaling integrator
ENSBTAG00000015567	7	15770431-16770431	16680584-16695716	TIMM44	translocase of inner mitochondrial membrane 44
ENSBTAG00000015793	29	7127813-8127813	7501290-7566916	RAB38	"RAB38
ENSBTAG00000015837	1	116417195-117417195	116694941-116747192	P2RY12	purinergic receptor P2Y12
ENSBTAG00000017505	4	115952624-116952624	116913457-116954800	PAXIP1	PAX interacting protein 1
ENSBTAG00000017613	18	64431617-65431617	64698070-64705250	ZNF419	zinc finger protein 419
ENSBTAG00000017872	18	64431617-65431617	64520900-64525539	ZNF304	zinc finger protein 304
ENSBTAG00000018894	7	15770431-16770431	16347652-16361803	PEX11G	peroxisomal biogenesis factor 11 gamma
ENSBTAG00000020657	27	16331562-17331562	16420193-16546078	FAT1	FAT atypical cadherin 1
ENSBTAG00000020990	1	116417195-117417195	116847497-116879544	P2RY14	purinergic receptor P2Y14
ENSBTAG00000021703	1	116417195-117417195	116654940-117017533	MED12L	mediator complex subunit 12L
ENSBTAG00000021941	4	115952624-116952624	116115071-116898738	DPP6	dipeptidyl peptidase like 6
ENSBTAG00000021944	10	43913775-44913775	44831891-44846457	RTRAF	RNA transcription"
ENSBTAG00000021945	10	43913775-44913775	44846568-44939145	NID2	nidogen 2
ENSBTAG00000023445	1	116417195-117417195	117361864-117380735	SIAH2	siah E3 ubiquitin protein ligase 2

ENSBTAG00000026088	5	44288183-45288183	44365581-44371561	LYZ2	lysozyme C-2
ENSBTAG00000026323	5	44288183-45288183	44347280-44352877	LYSB	intestinal lysozyme
ENSBTAG00000026779	5	44288183-45288183	44506989-44515796	LYZ	lysozyme
ENSBTAG00000027980	20	9387590-10387590	10375865-10379392	TAF9	TATA-box binding protein associated factor 9
ENSBTAG00000027983	20	9387590-10387590	10193836-10223904	GTF2H2	"general transcription factor IIH
ENSBTAG00000030929	7	15770431-16770431	15992197-16008612	ZNF557	zinc finger protein 557
ENSBTAG00000031707	10	43913775-44913775	44297021-44564023	FRMD6	FERM domain containing 6
ENSBTAG00000031919	5	44288183-45288183	45047975-45065127	SLC35E3	solute carrier family 35 member E3
ENSBTAG00000032137	7	15770431-16770431	16399795-16424614	PNPLA6	patatin like phospholipase domain containing 6
ENSBTAG00000034360	20	9387590-10387590	10091779-10095638	SERF1A	small EDRK-rich factor 1A (telomeric)
ENSBTAG00000038151	7	15770431-16770431	15896010-15902152	ELOF1	elongation factor 1 homolog
ENSBTAG00000038926	18	64431617-65431617	64463752-64475319	ZNF805	zinc finger protein 805
ENSBTAG00000038947	18	64431617-65431617	65102323-65103788	ZSCAN4	zinc finger and SCAN domain containing 4
ENSBTAG00000039946	18	64431617-65431617	65375097-65405941	ZNF814	zinc finger protein 814
ENSBTAG00000040001	20	9387590-10387590	10317496-10341306	MARVELD2	MARVEL domain containing 2
ENSBTAG00000043369	5	44288183-45288183	44505113-44505215	U6	U6 spliceosomal RNA
ENSBTAG00000043658	18	64431617-65431617	65422830-65422949	5S_rRNA	5S ribosomal RNA
ENSBTAG00000043753	4	115952624-116952624	116495605-116495700	Vault	Vault RNA
ENSBTAG00000043777	7	15770431-16770431	15980316-15980445	U4	U4 spliceosomal RNA
ENSBTAG00000044018	7	15770431-16770431	16643893-16647693	PRR36	proline rich 36
ENSBTAG00000044160	20	9387590-10387590	9917221-9992861	MCCC2	methylcrotonoyl-CoA carboxylase 2
ENSBTAG00000044869	7	15770431-16770431	16711356-16711431	bta-mir-2455	bta-mir-2455
ENSBTAG00000044954	20	9387590-10387590	10246931-10247008	U6	U6 spliceosomal RNA
ENSBTAG00000046192	20	9387590-10387590	10375646-10389675	AK6	adenylate kinase 6
ENSBTAG00000046511	5	44288183-45288183	44392339-44400094	LYZ1	lysozyme (renal amyloidosis)

ENSBTAG00000046628	5	44288183-45288183	44423778-44430774	LYZ3	lysozyme 3
ENSBTAG00000046837	7	15770431-16770431	16386268-16388881	ZNF358	zinc finger protein 358
ENSBTAG00000046938	27	16331562-17331562	16383422-16406473	MTNR1A	melatonin receptor 1A
ENSBTAG00000047430	13	937036-1937036	1495902-1495984	bta-mir-2285m-1	bta-mir-2285m-1
ENSBTAG00000047486	20	9387590-10387590	9865315-9867404	CARTPT	CART prepropeptide
ENSBTAG00000048061	29	7127813-8127813	7007877-7197732	GRM5	glutamate metabotropic receptor 5
ENSBTAG00000048682	18	64431617-65431617	64609623-64619591	ZNF548	zinc finger protein 548
ENSBTAG00000048824	7	15770431-16770431	16474631-16476389	PET100	PET100 homolog
ENSBTAG00000048923	20	9387590-10387590	10263064-10263122	bta-mir-7858	bta-mir-7858
ENSBTAG00000049345	7	15770431-16770431	16712681-16712756	bta-mir-10178	bta-mir-10178
ENSBTAG00000049387	20	9387590-10387590	10264043-10264099	bta-mir-11986b	bta-mir-11986b
ENSBTAG00000050241	18	64431617-65431617	64880429-64880535	U6	U6 spliceosomal RNA
ENSBTAG00000051980	18	64431617-65431617	64842670-64842776	U6	U6 spliceosomal RNA

Supplementary table 2. List of quantitative trait loci (QTL) within 1Mb windows for each region indicated by GWAS.

Chr	Windows (start-end)	QTL (start-end)	QTL ID	PubMEd ID	QTL Trait
1	116417195-117417195	116786785-116786789	66772	19966163	Body weight (yearling)
1	116417195-117417195	117284016-117284020	147015	29115939	Stillbirth (maternal)
1	116417195-117417195	117012745-117012749	155858	27506634	Milk tridecylic acid content
1	116417195-117417195	117406890-117406894	189070	31931702	Metabolic body weight
4	115952624-116952624	116287968-116287972	65435	26445451	Maturity rate
4	115952624-116952624	116162046-116162050	96731	26960806	Bovine tuberculosis susceptibility
4	115952624-116952624	116281770-116281774	176978	31299913	Conception rate
4	115952624-116952624	116309838-116309842	164246	30290764	Body weight (birth)
4	115952624-116952624	116604485-116604489	155862	27506634	Milk tridecylic acid content
4	115952624-116952624	116792818-116792822	151654	29163638	Muscle carnosine content
4	115952624-116952624	116803810-116803814	153963	29390120	Metabolic body weight
4	115952624-116952624	116878991-116878995	153964	29390120	Subcutaneous fat
4	115952624-116952624	116884219-116884223	160060	30229962	Bovine respiratory disease susceptibility
4	115952624-116952624	116287968-116287972	65178	26445451	Body weight (mature)
4	115952624-116952624	116420551-116420555	23219	24205251	Average daily gain
4	115952624-116952624	116420551-116420555	37036	25273628	Lean meat yield
4	115952624-116952624	116515628-116515632	57571	26020874	Bovine respiratory disease susceptibility
4	115952624-116952624	116622078-116622082	31727	24733441	Body weight (birth)
4	115952624-116952624	116622078-116622082	31731	24733441	Body weight (weaning)
5	44288183-45288183	45222815-45222819	67072	19966163	Body weight (yearling)
5	44288183-45288183	44518543-44518547	71490	23785023	Inhibin level
5	44288183-45288183	45060597-45060601	138764	28852499	Scrotal circumference
5	44288183-45288183	44645933-44645937	174403	31139206	Milk yield
5	44288183-45288183	44645933-44645937	175391	31139206	Milk protein yield
5	44288183-45288183	44645933-44645937	175699	31139206	Somatic cell score

5	44288183-45288183	44645933-44645937	37110	25273628	Lean meat yield
5	44288183-45288183	44731189-44731193	25815	22449276	Milk fat yield
5	44288183-45288183	44804562-44804566	36563	24671577	Conception rate
5	44288183-45288183	44884870-44884874	32507	24858810	Milk myristic acid content
7	15770431-16770431	16314897-16314901	114127	27485317	Milk beta-lactoglobulin percentage
7	15770431-16770431	16530065-16530069	67856	19966163	Body weight (yearling)
7	15770431-16770431	16530065-16530069	67857	19966163	Body weight gain
7	15770431-16770431	16553269-16553273	106796	27209127	Conception rate
7	15770431-16770431	15823599-15823603	167179	30644110	Residual feed intake
7	15770431-16770431	16077313-16077317	154131	29459679	Stature
7	15770431-16770431	16182506-16182510	157436	29705414	Milking speed
7	15770431-16770431	16269011-16269015	152766	29380492	Fat area to ribeye area ratio
7	15770431-16770431	16387821-16387825	155625	27506634	Milk tridecylic acid content
7	15770431-16770431	16755539-16755543	151879	29163638	Intramuscular fat
7	15770431-16770431	15823599-15823603	21053	22497295	Residual feed intake
7	15770431-16770431	16182506-16182510	42792	21831322	Calving ease (maternal)
7	15770431-16770431	16182506-16182510	42793	21831322	Dairy form
7	15770431-16770431	16182506-16182510	42794	21831322	Daughter pregnancy rate
7	15770431-16770431	16182506-16182510	42795	21831322	Stillbirth (maternal)
7	15770431-16770431	16182506-16182510	42796	21831322	Foot angle
7	15770431-16770431	16182506-16182510	42797	21831322	Feet and leg conformation
7	15770431-16770431	16182506-16182510	42798	21831322	PTA type
7	15770431-16770431	16182506-16182510	42799	21831322	Teat placement - front
7	15770431-16770431	16182506-16182510	42800	21831322	Udder attachment
7	15770431-16770431	16182506-16182510	42801	21831322	Net merit
7	15770431-16770431	16182506-16182510	42802	21831322	Length of productive life
7	15770431-16770431	16182506-16182510	42803	21831322	Rear leg placement - side view
7	15770431-16770431	16182506-16182510	42804	21831322	Udder height
7	15770431-16770431	16182506-16182510	42805	21831322	Rump width

7	15770431-16770431	16182506-16182510	42806	21831322	Calving ease
7	15770431-16770431	16182506-16182510	42807	21831322	Somatic cell score
7	15770431-16770431	16182506-16182510	42808	21831322	Stillbirth
7	15770431-16770431	16182506-16182510	42809	21831322	Strength
7	15770431-16770431	16182506-16182510	42810	21831322	Teat length
7	15770431-16770431	16182506-16182510	42811	21831322	Udder depth
10	43913775-44913775	44326477-44326481	130863	28521758	Metabolic body weight
10	43913775-44913775	44720990-44720994	126278	28193175	Milk trans-10-Octadecenoic acid content
10	43913775-44913775	44720990-44720994	126293	28193175	Milk linoleic acid content
10	43913775-44913775	44720990-44720994	126371	28193175	Milk omega-6 fatty acid content
10	43913775-44913775	44720990-44720994	126375	28193175	Milk polyunsaturated fatty acid content
10	43913775-44913775	44639661-44639665	116745	27485317	Milk glycosylated kappa-casein percentage
10	43913775-44913775	44722169-44722173	163653	29391528	Milk caproic acid content
10	43913775-44913775	43996213-43996217	68150	19966163	Body weight gain
10	43913775-44913775	44021569-44021573	36034	25497826	Hoof and leg disorders
10	43913775-44913775	44431415-44431419	44720	21831322	Dairy form
10	43913775-44913775	44431415-44431419	44721	21831322	Daughter pregnancy rate
10	43913775-44913775	44431415-44431419	44722	21831322	Net merit
10	43913775-44913775	44431415-44431419	44723	21831322	Length of productive life
10	43913775-44913775	44431415-44431419	44724	21831322	Milk protein percentage
10	43913775-44913775	44431415-44431419	44725	21831322	Calving ease
10	43913775-44913775	44431415-44431419	44726	21831322	Somatic cell score
10	43913775-44913775	44431415-44431419	44727	21831322	Stillbirth
10	43913775-44913775	44752821-44752825	25900	22449276	Milk fat yield
13	937036-1937036	1392609-1392613	136242	28711251	Somatic cell score
13	937036-1937036	1392609-1392613	136243	28711251	Milk rennet coagulation time
13	937036-1937036	1754931-1754935	136297	28711251	Milk protein-to-fat ratio

13	937036-1937036	1219215-1219219	46606	21831322	Calving ease (maternal)
13	937036-1937036	1219215-1219219	46607	21831322	Foot angle
13	937036-1937036	1219215-1219219	46608	21831322	Feet and leg conformation
13	937036-1937036	1219215-1219219	46609	21831322	PTA type
13	937036-1937036	1219215-1219219	46610	21831322	Udder attachment
13	937036-1937036	1219215-1219219	46611	21831322	Milk fat yield
13	937036-1937036	1219215-1219219	46612	21831322	Milk yield
13	937036-1937036	1219215-1219219	46613	21831322	Net merit
13	937036-1937036	1219215-1219219	46614	21831322	Milk protein yield
13	937036-1937036	1219215-1219219	46615	21831322	Rear leg placement - rear view
13	937036-1937036	1219215-1219219	46616	21831322	Rear leg placement - side view
13	937036-1937036	1219215-1219219	46617	21831322	Teat placement - rear
13	937036-1937036	1219215-1219219	46618	21831322	Udder height
13	937036-1937036	1219215-1219219	46619	21831322	Calving ease
13	937036-1937036	1437034-1437038	168544	30838020	Milk beta-casein percentage
13	937036-1937036	1692483-1692487	146250	29178833	Stillbirth (maternal)
18	64431617-65431617	65093667-65093671	130674	27889122	Curd water yield
18	64431617-65431617	65027526-65027530	130809	28521758	Average daily gain
18	64431617-65431617	64802282-64802286	69744	25989905	Milk zinc content
18	64431617-65431617	65431145-65431149	123847	27889128	Length of productive life
18	64431617-65431617	65372818-65372822	65281	26445451	Body weight (mature)
18	64431617-65431617	65224708-65224712	173274	31633203	Udder width
18	64431617-65431617	65227494-65227498	173295	31633203	Conformation score
18	64431617-65431617	64774968-64774972	181361	31718557	Conception rate
18	64431617-65431617	64466543-64466547	49955	21831322	Calving ease (maternal)
18	64431617-65431617	64466543-64466547	49956	21831322	Dairy form
18	64431617-65431617	64466543-64466547	49957	21831322	Daughter pregnancy rate
18	64431617-65431617	64466543-64466547	49958	21831322	Stillbirth (maternal)
18	64431617-65431617	64466543-64466547	49959	21831322	Length of productive life

18	64431617-65431617	64466543-64466547	49960	21831322	Rump angle
18	64431617-65431617	64466543-64466547	49961	21831322	Teat length
18	64431617-65431617	64586972-64586976	37183	25273628	Lean meat yield
18	64431617-65431617	65425119-65425123	35028	25511820	Milk linoleic acid content
20	9387590-10387590	10266516-10266520	69030	19966163	Body weight (yearling)
20	9387590-10387590	10266516-10266520	69031	19966163	Body weight gain
20	9387590-10387590	10085177-10085181	65600	26445451	Maturity rate
20	9387590-10387590	10243096-10243100	122371	26923315	Daughter pregnancy rate
20	9387590-10387590	10243096-10243100	122405	26923315	Conception rate
20	9387590-10387590	10243096-10243100	127040	28259397	Inseminations per conception
20	9387590-10387590	10243096-10243100	127057	28259397	Calving to conception interval
20	9387590-10387590	9407302-9407306	30592	22921614	Sire conception rate
20	9387590-10387590	9407302-9407306	30595	22921614	Fertilization rate
20	9387590-10387590	9407302-9407306	30596	22921614	Early embryonic survival
20	9387590-10387590	9539933-9539937	50476	21831322	Body depth
20	9387590-10387590	9539933-9539937	50477	21831322	Calving ease (maternal)
20	9387590-10387590	9539933-9539937	50478	21831322	Daughter pregnancy rate
20	9387590-10387590	9539933-9539937	50479	21831322	Foot angle
20	9387590-10387590	9539933-9539937	50480	21831322	Feet and leg conformation
20	9387590-10387590	9539933-9539937	50481	21831322	Milk fat percentage
20	9387590-10387590	9539933-9539937	50482	21831322	PTA type
20	9387590-10387590	9539933-9539937	50483	21831322	Teat placement - front
20	9387590-10387590	9539933-9539937	50484	21831322	Udder attachment
20	9387590-10387590	9539933-9539937	50485	21831322	Milk fat yield
20	9387590-10387590	9539933-9539937	50486	21831322	Milk yield
20	9387590-10387590	9539933-9539937	50487	21831322	Net merit
20	9387590-10387590	9539933-9539937	50488	21831322	Length of productive life
20	9387590-10387590	9539933-9539937	50489	21831322	Milk protein percentage
20	9387590-10387590	9539933-9539937	50490	21831322	Milk protein yield

20	9387590-10387590	9539933-9539937	50491	21831322	Rear leg placement - rear view
20	9387590-10387590	9539933-9539937	50492	21831322	Rear leg placement - side view
20	9387590-10387590	9539933-9539937	50493	21831322	Rump width
20	9387590-10387590	9539933-9539937	50494	21831322	Calving ease
20	9387590-10387590	9539933-9539937	50495	21831322	Somatic cell score
20	9387590-10387590	9539933-9539937	50496	21831322	Stillbirth
20	9387590-10387590	9539933-9539937	50497	21831322	Stature
20	9387590-10387590	9539933-9539937	50498	21831322	Strength
20	9387590-10387590	9539933-9539937	50499	21831322	Udder depth
20	9387590-10387590	9586885-9586889	16047	20665291	Fat percentage
20	9387590-10387590	9586885-9586889	16302	20665291	Intramuscular fat
20	9387590-10387590	9594578-9594582	64398	25771056	Milk riboflavin content
20	9387590-10387590	9802405-9802409	37289	25273628	Carcass weight
20	9387590-10387590	10243096-10243100	154013	29390120	Residual feed intake
20	9387590-10387590	10254654-10254658	154213	29459679	Stature
20	9387590-10387590	9992985-9992989	50512	21831322	Udder height
20	9387590-10387590	10038682-10038686	22615	23031337	Body weight
20	9387590-10387590	10063469-10063473	50522	21831322	Stillbirth (maternal)
20	9387590-10387590	10223690-10223694	63733	26286463	Reproductive efficiency
20	9387590-10387590	10223690-10223694	63760	26286463	Age at first calving
20	9387590-10387590	10243096-10243100	57129	23759029	Daughter pregnancy rate
20	9387590-10387590	10243096-10243100	57166	23759029	Conception rate
20	9387590-10387590	10243096-10243100	57200	23759029	Length of productive life
20	9387590-10387590	10243096-10243100	57232	23759029	Net merit
20	9387590-10387590	10243096-10243100	57261	23759029	Milk fat percentage
20	9387590-10387590	10261742-10261746	28509	23736061	Residual feed intake
27	16331562-17331562	17229316-17229320	69398	19966163	Body weight gain
27	16331562-17331562	16616482-16616486	70382	25989905	Milk potassium content
27	16331562-17331562	16579768-16579772	53392	21831322	Calving ease (maternal)

27	16331562-17331562	16579768-16579772	53393	21831322	Dairy form
27	16331562-17331562	16579768-16579772	53394	21831322	Daughter pregnancy rate
27	16331562-17331562	16579768-16579772	53395	21831322	Foot angle
27	16331562-17331562	16579768-16579772	53396	21831322	Milk fat percentage
27	16331562-17331562	16579768-16579772	53397	21831322	Milk fat yield
27	16331562-17331562	16579768-16579772	53398	21831322	Milk yield
27	16331562-17331562	16579768-16579772	53399	21831322	Net merit
27	16331562-17331562	16579768-16579772	53400	21831322	Length of productive life
27	16331562-17331562	16579768-16579772	53401	21831322	Milk protein percentage
27	16331562-17331562	16579768-16579772	53402	21831322	Milk protein yield
27	16331562-17331562	16579768-16579772	53403	21831322	Rear leg placement - side view
27	16331562-17331562	16579768-16579772	53404	21831322	Calving ease
27	16331562-17331562	16579768-16579772	53405	21831322	Stillbirth
27	16331562-17331562	16579768-16579772	53406	21831322	Strength
27	16331562-17331562	16579768-16579772	53407	21831322	Udder cleft
27	16331562-17331562	17149373-17149377	23935	23851991	Average daily gain
29	7127813-8127813	7209177-7209181	116596	27485317	Milk glycosylated kappa-casein percentage
29	7127813-8127813	7209177-7209181	112228	27485317	Milk kappa-casein percentage
29	7127813-8127813	7174009-7174013	173334	31633203	Rump angle
29	7127813-8127813	7343995-7343999	155596	27506634	Milk tridecylic acid content
29	7127813-8127813	7809271-7809275	181416	31718557	Conception rate
29	7127813-8127813	7809271-7809275	181558	31718557	Inseminations per conception

CHAPTER 3 – Genomics studies for *Anaplasma marginale* infection level in Braford and Hereford cattle

ABSTRACT - Bovine anaplasmosis is a bacterial infection caused by rickettsia *Anaplasma marginale* whose clinical signs include weight loss, anemia and can lead to death. Rickettsia is also part of the parasitic complex known as Bovine Parasitic Sadness (BPS), along with the protozoa *Babesia bovis* and *Babesia bigemina*. Despite the economic importance of anaplasmosis, there is no genomic study for this trait reported in the literature. Thus, the objectives of this work were to estimate variance components for levels of infection by *A. marginale* and their genetic correlation with levels of infection by *B. bigemina*, *B. bovis,* and tick count, calculate the prediction accuracy for *A. marginale* and conduct a genome-wide association study (GWAS). For that, information from single nucleotide polymorphisms (SNPs) imputed to high density (~ 777,000 markers) was used. The phenotype was based on the quantification of the number of copies of *A. marginale* in the blood tissue of 1,370 Braford and 153 Hereford cattle by the quantitative PCR technique. The variance components were estimated by Bayesian analysis using a multivariate model including phenotypes information of infection levels of *B. bigemina* (n=1,880), *B. bovis* (1,858)*,* and tick count (n=5,867). The genomic best linear unbiased prediction method was used to obtain genomic estimated breeding values (GEBV) and to perform GWAS. The outcomes indicated low heritability (0.090) and low prediction accuracy (0.180 ± 0.050) for levels of infection by *A. marginale*. The genetic correlations between the infection by *A. marginale* and *B. bigemina* was high (0.793), in contrasts with estimates between *A. marginale* infection with *B. bovis* and tick count measures which were close to zero. The top 10 regions of GWAS are located on chromosomes 2, 5, 8, 10, 13, 15, 17, 20, 24, and 29. In these locations, candidate genes related to the interaction between host and pathogen and response to inflammation were found. In conclusion, despite the low heritability, we identified evidence of genetic variation in *A. marg*inale infections between cattle breeds. The GWAS detected genes and QTL associated with the immune response, which may guide future studies with a larger sample size. Thus, the results obtained in this study can contribute to the establishment of strategies for selecting animals capable of maintaining lower levels of infections caused by BPS.

Keywords: Anaplasmosis, parasitemia, prediction, rickettsia.

3.1. Introduction

Bovine anaplasmosis is a bacterial infection caused by rickettsia *Anaplasma marginale* that affects the red cells of animals, whose clinical cases are characterized by severe anemias (Kocan et al., 2003). Anaplasmosis is one of the most common bovine diseases in tropical and subtropical regions, causing economic losses due to expenses with treatment of the disease and death of susceptible animals (Dumler et al., 2001; Kocan et al., 2003).

The principal vector of the disease in Brazil is the tick *Rhipicephalus microplus,* which transmits rickettsia along with saliva during blood-sucking (Ribeiro; Lima, 1995). Although the pathogen has been identified in other ruminants, such as buffalo, for example, *A. marginale* is a preferred parasite of cattle (Obregón et al., 2019). In contrast to other infections caused by bacteria, such as bovine paratuberculosis, calves are considered more resistant to severe cases of anaplasmosis, as they are protected by colostral antibodies. Animals with *A. marginale* remain asymptomatic for an average of 28 days. After this period, symptoms such as fever, weight loss, abortion, lethargy, anemia, and eventually death occur (Kocan et al., 2010).

In addition to the damage caused individually, rickettsia also integrates the parasitic complex known as Bovine Parasitic Sadness (BPS), together with the protozoa that cause babesiosis (*Babesia bovis* and *Babesia bigemina*). The infectious disease process caused by the three pathogens are similar in cattle, which infected animals once recovered, become persistent carriers of parasites and sources of contamination for other ticks (Ribeiro; Lima, 1996; Kocan et al., 2004).

Animals of indicine breeds (*Bos taurus indicus*) are more resistant to ectoparasites and hemoparasites transmitted by ticks, when compared to taurines (*Bos taurus taurus*) (Silva et al., 2002; de Alencar, 2004), which is one of the main reasons for the predominance of indicine breeds in Brazil. However, meat from taurine animals has better quality, especially due to its greater tenderness and marbling. Given this, the use of crossbred cattle has been increasing in regions where animals are raised in the pasture with the presence of ticks and diseases transmitted by them. Another strategy for controlling parasitic diseases can be achieved by selecting resistant animals (Giglioti et al., 2018a).

The objective of this work was to evaluate the possibility of obtaining selection gains for low levels of *A. marginale* infection (AmIL) in Braford and Hereford animals,

by estimating the variance components for AmIL, phenotypic and genetic correlation between AmIL, tick count, levels of infection by *B. bovis* (BovIL), and *B. bigemina* (BigIL). Also, the genomic prediction accuracy for AmIL was calculated and the genome-wide association study (GWAS) was employed, looking for regions of the genome associated with AmIL.

3.2. Material and methods

3.2.1. Animals and phenotypic data

Phenotypic data were obtained from 1,370 Braford and 153 Hereford cattle raised in five farms located in the Rio Grande do Sul state, Brazil. These animals belong to the animal breeding program Delta G (Gensys Associated Consultants, RS, Brazil). They were born in the same season and year (spring 2008), distributed in contemporary groups (CG) formed by farm and management groups. The protocol for quantifying the number of copies of *A. marginale* in the blood tissue of the animals was carried out according to the methodology proposed by Buling et al. (2007) and adapted by Giglioti et al. (2018b).

DNA extraction was performed with Easy-DNA ™ kit (Invitrogen USA, catalog number K180001) and quantification of the number of copies of *A. marginale* by qPCR technique. The primers used to flank a fragment of 118 nucleotides located in the *major surface protein 1b* gene (*MSP1b*, Genbank accession number: M59845.1). The samples were subjected to qPCR together with control and dilutions of the synthetic DNA gBlocks® Gene Fragments (IDT, Coralville, IA, USA) which contains the known concentration of the *MSP1b* sequence. BioRad's CFX96 software was used to convert the light signals produced at each reaction cycle into quantitative values for the target DNA, with the baseline threshold adjusted to 150 fluorescence units.

Genetic correlations were estimated using phenotypes of BigIL (n = 1,880), BovIL (n = 1,858) and tick count (n=5,867), descriptive statistics are showed in Table 1. The levels of infection of the protozoa (*B. bigemina* and *B. bovis*) were evaluated following the same methodology described for *A. marginale* using the primers described by Buling et al. (2007). One to three tick counts were performed, considering only parasites larger than 4.5 mm on the animals' right side, as proposed by Hermans et al. (1994) and Utech et al. (1978). Counts were done manually when all bovines into a contemporary group (animals raised together, receiving the same feeding and

sanitary management) presented more than 20 engorged females with a spacing of at least 30 days. The phenotypic values were submitted to a logarithmic transformation to obtain an approximation of the normal curve, and the descriptive statistics of the phenotypes were performed in the software R v.4.0.3 (Team, 2013).

Table 1. Descriptive statistics with the standard deviation (in parenthesis) of the copy number of pathogens *A. marginale* (AmIL), *B. bigemina* (BigIL), and *B. bovis* (BovIL) in Braford and Hereford breeds.

	AmIL		**BigIL**		**BovIL**	
	Braford	**Hereford**	**Braford**	**Hereford**	**Braford**	**Hereford**
Minimum	0,0	0,0	0,0	0,0	0,0	0,0
Median	3,603	3,942	1,735	2,207	1,979	1,961
Mean	3,191 (1,26)	3,479 (1,30)	1,480 (1,075)	2,094 (1,086)	1,683 (1,084)	1,724 (1,252)
Maximum	5,416	5,688	4,08	4,127	5,188	4,147

3.2.2. Genotypic data

We used genotypic information from 3,977 animals originaly genotyped in panels of 27K (n=47), 30K (n=122), 50K (n=3,647), 150K (n=160), and HD (n=1) and imputed to a high-density panel (~777,000 SNPs) using Beagle v software. 5.1 (Browning; Zhou; Browning, 2018), where 1,412 animals (115 Hereford, 113 Braford, and 1,184 Nelore) genotyped on Illumina BovineHD Beadchip panel (Illumina Inc., San Diego, CA, USA) were used as a reference population.

The SNP map used in the analysis was updated from UMD v3.1 assembly (Kinsella et al., 2011) to ARS-UCD1.2 coordinates (available from https://www.animalgenome.org/repository/cattle/UMC_bovine_coordinates/). The accuracy imputation obtained by the rate of genotypes correctly imputed was 0.97 (more details in chapter 2). Genotype data were submitted to quality control (QC) before and after imputation.

The parameters of exclusion used for QC before imputation were: SNP with a call rate ≤ 0.95, minor allele frequency (MAF) ≤ 2%, P-value in the Hardy-Weinberg equilibrium test < 10-5, non-autosomal markers, and different SNPs mapped to the same genomic coordinate. The exclusion criteria for samples were call rate ≤ of 0.90 and minimum CG size of four animals. QC procedures after imputation were the same

described above but without the Hardy-Weinberg equilibrium parameter. QC of markers and samples were performed through Plink v.1.9 (Chang et al., 2015). After the QC, 502,398 SNP and 3,977 animals remained for the next analyses.

Although the Braford breed was originally formed by the composition of 3/8 indicine breed and 5/8 Hereford breed, in Brazil there are variations in the ancestral proportions of the animals. Thus, the LAMP-ANC v. 2.5 (Sankararaman et al., 2008) was applied to estimate individual genomic heterozygosity. It was considered known ancestry, using genotypic information from 2,376 animals (1,184 Nelore and 1,192 Hereford) as reference individuals. The output obtained from LAMP-ANC coded a single locus as 0, 0.5, or 1, where 0.5 represents the heterozygous genotype. When both alleles came from different ancestries (0.5 in each allele corresponding to the same locus) were computed as 1, and alleles that came from the same ancestral was 0, the sum of these results was divided by the total of SNPs; following the methodology applied by Khayatzadeh et al. (2019).

3.2.3. Components of variance

The components of variance were estimated by a Bayesian approach, using the Markov chain Monte Carlo framework and Gibbs sampler algorithm implemented in gibbs2f90 software (Misztal et al., 2002). We used a multivariate model with the initial values of estimates obtained by the AIREMLF90 software (Misztal et al., 2002). A total of 500,000 iterations were performed, of which 50,000 were removed as burn-in, and one for every 10 samples was taken for further analysis, thus avoiding highly correlated samples. The model used was:

$$y = Xb + Zu + Pp + e \quad [1]$$

where ***y*** is the vector of phenotypes, ***b*** is the vector of fixed effects (CG) and heterozygosity as covariate, ***u*** is the vector of additive genetic value per individual and, ***p*** is a vector of permanent environment only considered for tick count, ***e*** is the vector of residual effects, ***X***, ***Z*****,** and ***P*** are incidence matrices that associate ***b***, ***u***, and ***p*** to observations, respectively. Genetic and residual values were assumed to have a multivariate normal distribution $P(u|\ \sigma_u^2) \sim N(0, G\sigma_u^2)$ and $P(e|\ \sigma_e^2) \sim N(0, \boldsymbol{I}\sigma_e^2)$. The genomic relationship matrix (***G***) was built according to VanRaden (2008), in which $G=ZZ'/2\sum p_k\ q_k$, with *p* and *q* being the allele frequencies of marker *k*. The phenotypic

correlations between the traits were calculated using Pearson's correlation in the software R.

3.2.4. Genomic prediction and GWAS

The method employed to obtain the genomic prediction breeding value (GEBV) was the genomic best linear unbiased prediction (GBLUP) using a multivariate model as described in equation [1] implemented in the BLUPF90 software (Misztal et al., 2002).

For the cross-validation process, the sample group was randomized and divided into five groups of approximately 304 animals (corresponding to 20% of the studied population). When one set of data had its phenotype masked (replaced by lost information) the other four were used as a training group, and this procedure was repeated 10 times. Accuracy was calculated using Pearson's correlation between the estimated GEBV and the phenotype adjusted for fixed effects, divided by the square root of AmIL heritability.

GWAS was performed for AmIL using weighted genomic best linear unbiased predictor (WGBLUP) with 2 iterations, adopting the following univariate model:

$$y = Xb + Zu + e \quad [2]$$

where ***y*** is the vector of phenotypes, ***b*** is the vector of fixed effects (farm and management group) and covariates of genomic heterozygosity, ***u*** is the vector of individual genetic value, and ***e*** is the residual effect. The random effects were assumed to be $\boldsymbol{u} \sim N(0, \mathbf{G}\sigma_u^2)$ and $\boldsymbol{e} \sim N(0, \mathbf{I}\sigma_e^2)$. ***X*** and ***Z*** are incidence matrices that associate ***b*** and ***u*** with observations. The effects of SNPs were estimated based on GEBV using postGSf90 software.

After GWAS, regions within 500 kb on either side of the top ten SNP of the greatest variance were analyzed using the Ensembl BioMart tool (Kinsella et al., 2011). The CattleQTL database r.42 (Hu et al., 2013) were used to detect genes and QTL located in the selected regions. The coordinates of the genes were based on the assembly of the bovine genome ARS-UCD1.2. The functional relationship between the genes and AmIL phenotype was examined in the literature.

3.3. Results e Discussion

The copy numbers of *A. marginale* were higher than the registered values for *B. bovis* and *B. bigemina* (Table 1). According to Giglioti et al (2018a) this difference can express the different moments of action of the parasites. *Babesia* spp reach the peak of infection around 12 days after the tick has attached to the cow, while *A. marginale* only reach the peak of infection after 28 days and decreases its presence in the red cells more slowly compared to *Babesia* spp (Giglioti et al., 2018a).

The heritability estimate for levels of infection of *A. marginale* was 0.090 (Table 2). Traits related to immune responses commonly have low heritability, such as passive immune response trait (Johnston et al., 2020), *Babesia bovis* infection levels (Cavani et al., 2020) and *B. bigemina* infection levels (chapter 2) with heritability of 0.090, 0.07 and 0.090, respectively.

Table 2. Heritability (diagonal), genetic correlations (above the diagonal), limits of 95% for the highest density posterior interval (in parenthesis), and phenotypic correlation (below the diagonal).

Trait	*A. marginale*	*B. bigemina*	*B. bovis*	Tick count
A. marginale	**0.090** (0.033; 0.150)	0.793 (0.582; 0.948)	0.043 (-0.459; 0.586)	-0.019 (-0.271; 0.240)
B. bigemina	0.330	**0.094** (0.039; 0.156)	0.524 (0.129; 0.852)	0.239 (0.027; 0.461)
B. bovis	0.209	0.172	**0.099** (0.044; 0.160)	0.160 (-0.244; 0.217)
Tick count	-	-	-	**0.247** (0.215; 0.279)

The genetic parameters estimated for the phenotypes of infection by *B. bovis* and tick count were similar to those found by Cavani et al. (2020), that used the same sample group, but with a bivariate model and genotypes with 50K of density. According Jia and Jannink (2012) the mutivariate model can increase estimates of heritability when there is a correlation between traits and at least one has high heritability. Although there was no increase in estimates, the intervals at a later time were shorter compared to those reported by Cavani and in the chapter 2 for *B. bigemina*.

Although of the small magnitude of heritability, results indicated that there is genetic variation in the trait according to the breed of the animal. For instance, the mean of anaplasmosis parasitemia in Hereford was higher than found in Braford animals (P<0.001). The individual heterozygosity calculated for Braford was 0.46

(±0.09), Menegassi et al. (2018) found similar values in animals of the same breed (0.49).

We found a strong genetic correlation between AmIL and BigIL (0.793) (Table 2). The correlations values can indicate similarity between reactions to inflammatory processes triggered by increasing the susceptibility of erythrocytes to oxidative damage caused by pathogens. Esmaeilnejad et al. (2018) identified that *B. bigemina* and *A. marginale* act to reduce the availability of trace elements such as copper, zinc, manganese and selenium, which make up antioxidant enzymes evolved on the immune response against parasites.

The genetic correlations estimated between infection level of *A. marginale* with *B. bovis* and tick count were close to zero. Since there is no genetic correlations information for AmIL reposted in the literature, Giglioti et al. (2018a) found phenotypic correlations close to zero between *Babesia* spp and *A. marginale*, and also between *Babesia* spp measured in Canchim cattle. In contrasts, Giglioti et al. (2020) identified a high phenotypic correlation between *B. bovis* and *B. bigemina* infections (0.75) in Holstein. Considering that the correlation is a population measurement, intrinsic factors of the breeds and age of the animals during the collection period may have contributed to the differences found in the present study (Pearson, 1909; Giglioti et al., 2020).

The accuracy prediction for AmIL was low (0,188±0,050), which was expected due to the low heritability of the studied phenotype. Traits related to disease resistance are complex and require a large sample size to achieve accurate results (Dehnavi et al., 2018; Johnston et al., 2020). *A. marginale* infection levels is a polygenic trait, which can be observed by the peaks in different chromosomes in the Figure 1.

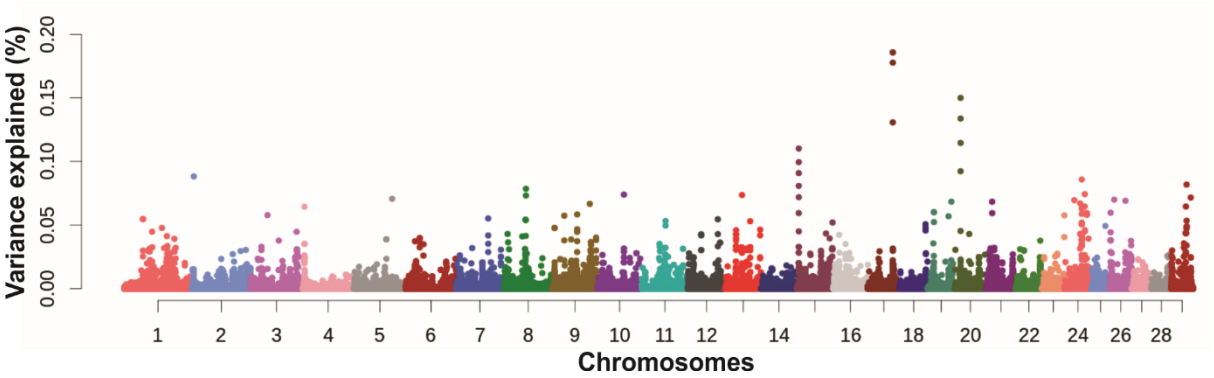

Figure 1. Manhattan plot of GWAS for *A. marginale* infection level with the highest variance explained located in chromosomes autosomes 2, 5, 8, 10, 13, 15, 17, 20, 24, and 29.

The regions of the top 10 SNP of higher variance from GWAS was in overlap with 93 regions containing 67 genes (Supplementary table 1) and 121 QTLs (supplementary table 2). It is interesting the presence of QTL previously associated with susceptibility to bovine tuberculosis, because anaplasmosis and tuberculosis are caused by bacterias that trigger inflammatory responses mediated by TH1 (T-helper-cell) (Huygen et al., 1994). QTL for somatic cell score was also previews reported on the explored regions, related to increased concentration of immune cells in milk, typically attributed for inflammation of the mammary glands by bacteria that cause mastitis (Shook; Schutz, 1994).

According to Lahmers et al. (2005), T cells play an important role in defending against *A. marginale*, where they are activated in response to contact with proteins MSP2 in the rickettsia membrane. In calves, a high concentration of circulating T cells occurs naturally, which trigger reactions that prevent the proliferation of infected cells, making young animals less susceptible to the development of anaplasmosis.

Given the relevance of the T cells immune response against *A. Marginale*, the genes *KSR2* and *NFRKB* showed in Table 3, are promising because they have been associated with cell activation and differentiation from T to Th1 and Th2 in immune response against antigens (Mirza et al., 2011; Xue et al., 2013). The gene *NOS1* was associated with inflammatory response in rats (Baig et al., 2015), respiratory diseases in cattle (Neupane et al., 2018), and participates in the metabolism of Nitric oxid evolved in response to *A. marginale* macrophage-mediated (Gale et al., 1997).

Table 3. Genes positioned into a windows around of the top ten regions indicated by GWAS related with immune response based on review of the literature.

Gene ID	Chr	Gene (start-end)	Gene Name	Description
ENSBTAG00000019036	24	39834619-39935989	*ARHGAP28*	Rho GTPase activating protein 28
ENSBTAG00000011661	10	61437230-61529573	*CEP152*	centrosomal protein 152
ENSBTAG00000032097	17	57900735-57938125	*FBXO21*	F-box protein 21
ENSBTAG00000013333	5	88602173-88658602	*GYS2*	glycogen synthase 2
ENSBTAG00000050824	2	4187288-4227153	*HS6ST1*	heparan sulfate6 O-sulfotransferase 1
ENSBTAG00000010530	5	88796314-88801862	*IAPP*	islet amyloid polypeptide
ENSBTAG00000044119	17	57231745-57664911	*KSR2*	kinase suppressor of ras 2
ENSBTAG00000017610	29	36070018-36099972	*NFRKB*	nuclear factor related to kappaB binding protein
ENSBTAG00000002023	17	57763560-57876534	*NOS1*	nitric oxide synthase 1
ENSBTAG00000002746	8	51153923-51214968	*OSTF1*	osteoclast stimulating factor 1
ENSBTAG00000017260	5	89133571-89283293	*PDE3A*	phosphodiesterase 3A
ENSBTAG00000008825	13	38533190-38546652	*POLR3F*	RNA polymerase III subunit F
ENSBTAG00000043976	17	58188410-58250340	*RNFT2*	"ring finger protein
ENSBTAG00000018295	13	38561341-38592220	*SEC23B*	SEC23 homolog B"
ENSBTAG00000019602	5	89019014-89099984	*SLCO1C1*	solute carrier organic anion transporter family member 1C1

Deficiency in the expression of *FBXO21* reduces production of proinflammatory cytokines and interferon-mediated immune response activity in rats. *SLCO1C1* expressed in brain endothelial cells, is involved in neuronal circuits associated with the immune system by activating interleukins that contribute to generate a complete response against the pathogen (Ridder et al., 2011).

In humans, the genes *CEP152, POLR3F, RNFT2,* and *IAPP* have been associated with an innate immune response (Westwell-Roper et al., 2016; Ma et al., 2019; Kodani et al., 2020; Tong et al., 2020), and the *SEC23B* is involved with viral immune response (Zeyen et al., 2020).

Karthikeyan et al (2019) and Zhao et al (2019) reported relationship of the expression of *HS6ST1* and *ARHGAP28* genes with pathogen host interaction. *PDE3A* was associated with heat tolerance in Holstein and with modulation of interaction between bacteria and host in humans (Dikmen et al., 2013; Weber et al., 2016). The expression of the gene *GYS2* was previously associated with pathways of presenting antigens by Dendritic cell (DC) activate by Toll-like receptor (TLR) (Thwe et al., 2017). In cattle, the gene *OSTF1* was associated with immune response to mastitis infection (Schwerin et al., 2003).

The top ten regions based on the GWAS results are in overlap with genes and QTL associated with an immune response, which can influence the variations in the levels of *A. marginale* infection observed between the Braford and Hereford breeds. Further studies with a larger sample group and the validation of the genes found are indicated. The future inclusion of the phenotype in breeding programs can be considered because although they present a low heritability, the additive genetic variance can change as changes in the allele frequencies occur as a result of the selection (Falconer, 1996).

3.4. Conclusion

Our results provide information about genetic aspects of AmIL reported for the first time in the present study. Although the small magnitude of heritability, there are indication of obtaining selection gains for AmIL. The moderate genetic correlations between the studied phenotypes indicate that quantifications for *A. marginale* can predict levels of infection for *B. bigemina*. It was possible to estimate the accuracy of prediction for levels of infection by *A. marginale*, however, the low heritability contributed to reduced accuracy.

The regions detected by GWAS analysis on chromosomes 2, 5, 8, 10, 13, 15, 17, 20, 24 and 29 may be a promising region for future studies associated with BigIL and contributed to the better understanding of the biological relationship between genetic factors and resistance to infection with *A. marginale* in cattle.

3.5. References

Baig MS, Zaichick S V, Mao M, De Abreu AL, Bakhshi FR, Hart PC, Saqib U, Deng J, Chatterjee S, Block ML (2015) NOS1-derived nitric oxide promotes NF-κB transcriptional activity through inhibition of suppressor of cytokine signaling-1. **Journal**

of Experimental Medicine, 212:1725–1738.

Browning BL, Zhou Y, Browning SR (2018) A one-penny imputed genome from next-generation reference panels. **The American Journal of Human Genetics**, 103:338–348.

Buling A, Criado-Fornelio A, Asenzo G, Benitez D, Barba-Carretero JC, Florin-Christensen M (2007) A quantitative PCR assay for the detection and quantification of Babesia bovis and B. bigemina. **Veterinary parasitology**, 147:16–25.

Cavani L, Braz CU, Giglioti R, Okino CH, Gulias-Gomes CC, Caetano AR, De Sena Oliveira MC, Cardoso FF, De Oliveira HN (2020) Genomic study of Babesia bovis infection level and its association with tick count in Hereford and Braford cattle. **Frontiers in Immunology**, 11:.

Chang CC, Chow CC, Tellier LCAM, Vattikuti S, Purcell SM, Lee JJ (2015) Second-generation PLINK: rising to the challenge of larger and richer datasets. **Gigascience**, 4:7.

De Alencar MM (2004) Perspectivas para o melhoramento genético de bovinos de corte no Brasil.

Dehnavi E, Mahyari SA, Schenkel FS, Sargolzaei M (2018) The effect of using cow genomic information on accuracy and bias of genomic breeding values in a simulated Holstein dairy cattle population. **Journal of dairy science**, 101:5166–5176.

Dikmen S, Cole JB, Null DJ, Hansen PJ (2013) Genome-wide association mapping for identification of quantitative trait loci for rectal temperature during heat stress in Holstein cattle. **PloS one**, 8:e69202.

Dumler JS, Barbet AF, Bekker CP, Dasch GA, Palmer GH, Ray SC, Rikihisa Y, Rurangirwa FR (2001) Reorganization of genera in the families Rickettsiaceae and Anaplasmataceae in the order Rickettsiales: unification of some species of Ehrlichia with Anaplasma, Cowdria with Ehrlichia and Ehrlichia with Neorickettsia, descriptions of six new species combi. **International journal of systematic and evolutionary microbiology**, 51:2145–2165.

Esmaeilnejad B, Tavassoli M, Samiei A, Hajipour N, Imani-Baran A, Farhang-Pajuh F (2018) Evaluation of oxidative stress and antioxidant status, serum trace mineral levels and cholinesterases activity in cattle infected with Anaplasma marginale. **Microbial pathogenesis**, 123:402–409.

Falconer DS (1996) **Introduction to quantitative genetics**. [s.l.] Pearson Education India,

Gale KR, Leatch G, Dimmock CM, Wood PR (1997) Anaplasma marginale: effect of the treatment of cattle with an interferon γ-neutralizing monoclonal antibody or the nitric oxide synthetase inhibitor aminoguanidine on the course of infection. **Parasite immunology**, 19:411–417.

Giglioti R, De Oliveira HN, Bilhassi TB, Portilho AI, Okino CH, Marcondes CR, De Sena Oliveira MC (2018a) Estimates of repeatability and correlations of

hemoparasites infection levels for cattle reared in endemic areas for Rhipicephalus microplus. **Veterinary parasitology**, 250:78–84.

Giglioti R, De Oliveira HN, Okino CH, De Sena Oliveira MC (2018b) qPCR estimates of Babesia bovis and Babesia bigemina infection levels in beef cattle and Rhipicephalus microplus larvae. **Experimental and Applied Acarology**, 75:235–240.

Giglioti R, De Oliveira HN, Gutmanis G, Luciani GF, Azevedo BT, De Carvalho Fiorin CF, De Andrade MF, Silva MAF, Vercesi Filho AE, Katiki LM (2020) Correlations and repeatability between Babesia spp. infection levels using two dairy cattle breeding systems. **Experimental and Applied Acarology**, 81:599–607.

Hermans D, Houwer J De, Eelen P (1994) The affective priming effect: Automatic activation of evaluative information in memory. **Cognition & Emotion**, 8:515–533.

Hu Z-L, Park CA, Wu X-L, Reecy JM (2013) Animal QTLdb: an improved database tool for livestock animal QTL/association data dissemination in the post-genome era. **Nucleic acids research**, 41:D871–D879.

Huygen K, Lozes E, Gilles B, Drowart A, Palfliet K, Jurion F, Roland I, Art M, Dufaux M, Nyabenda J (1994) Mapping of TH1 helper T-cell epitopes on major secreted mycobacterial antigen 85A in mice infected with live Mycobacterium bovis BCG. **Infection and immunity**, 62:363–370.

Jia Y, Jannink J-L (2012) Multiple-Trait Genomic Selection Methods Increase Genetic Value Prediction Accuracy. **Genetics**, 192:1513–1522. Disponível em: <https://pubmed.ncbi.nlm.nih.gov/23086217>.

Johnston D, Mukiibi R, Waters SM, McGee M, Surlis C, McClure JC, McClure MC, Todd CG, Earley B (2020) Genome wide association study of passive immunity and disease traits in beef-suckler and dairy calves on Irish farms. **Scientific Reports**, 10:1–10.

Karthikeyan R, Gayathri P, Gunasekaran P, Jagannadham M V, Rajendhran J (2019) Transcriptome responses of intestinal epithelial cells induced by membrane vesicles of Listeria monocytogenes unveil novel insights into the host-pathogen cross talk. **bioRxiv**, 679951.

Khayatzadeh N, Mészáros G, Utsunomiya YT, Schmitz-Hsu F, Seefried F, Schnyder U, Ferenčaković M, Garcia JF, Curik I, Sölkner J (2019) Genome-wide mapping of the dominance effects based on breed ancestry for semen traits in admixed Swiss Fleckvieh bulls. **Journal of dairy science**, 102:11217–11224.

Kinsella RJ, Kähäri A, Haider S, Zamora J, Proctor G, Spudich G, Almeida-King J, Staines D, Derwent P, Kerhornou A (2011) Ensembl BioMarts: a hub for data retrieval across taxonomic space. **Database**, 2011:.

Kocan KM, De La Fuente J, Guglielmone AA, Meléndez RD (2003) Antigens and alternatives for control of Anaplasma marginale infection in cattle. **Clinical microbiology reviews**, 16:698–712.

Kocan KM, De La Fuente J, Blouin EF, Garcia-Garcia JC (2004) Anaplasma marginale

(Rickettsiales: Anaplasmataceae): recent advances in defining host-pathogen adaptations of a tick-borne rickettsia. **Parasitology**, 129:S285.

Kocan KM, De La Fuente J, Blouin EF, Coetzee JF, Ewing SA (2010) The natural history of Anaplasma marginale. **Veterinary parasitology**, 167:95–107.

Kodani A, Knopp K, Di Lullo E, Retallack H, Kriegstein A, DeRisi J, Reiter J (2020) Zika virus alters centrosome organization to suppress the innate immune response. **bioRxiv**,

Lahmers KK, Norimine J, Abrahamsen MS, Palmer GH, Brown WC (2005) The CD4+ T cell immunodominant Anaplasma marginale major surface protein 2 stimulates γδ T cell clones that express unique T cell receptors. **Journal of leukocyte biology**, 77:199–208.

Ma D, Liu Q, Zhang M, Feng J, Li X, Zhou Y, Wang X (2019) iTRAQ-based quantitative proteomics analysis of the spleen reveals innate immunity and cell death pathways associated with heat stress in broilers (Gallus gallus). **Journal of proteomics**, 196:11–21.

Menegassi SRO, Pereira GR, Aguiar PRL, Pereira KSS, Koetz Junior C, Braccini Neto J, Peripolli V, Berlitz CGB, Barcellos JOJ (2018) Candidate genes related to reproductive traits of Hereford and Braford bulls. **Semina: ciências agrárias. Londrina, PR. Vol. 39, n. 3 (maio/jun. 2018), p. 1335-1350**,

Mirza N, Pollock K, Hoelzinger DB, Dominguez AL, Lustgarten J (2011) Comparative kinetic analyses of gene profiles of naive CD4+ and CD8+ T cells from young and old animals reveal novel age-related alterations. **Aging cell**, 10:853–867.

Misztal I, Tsuruta S, Strabel T, Auvray B, Druet T, Lee DH (2002) BLUPF90 and related programs (BGF90). In: Proceedings of the 7th world congress on genetics applied to livestock production, **Anais**...

Neupane M, Kiser JN, Team BRDCCAPR, Neibergs HL (2018) Gene set enrichment analysis of SNP data in dairy and beef cattle with bovine respiratory disease. **Animal genetics**, 49:527–538.

Obregón D, Cabezas-Cruz A, Armas Y, Silva JB, Fonseca AH, André MR, Alfonso P, Oliveira MCS, Machado RZ, Corona-González B (2019) High co-infection rates of Babesia bovis, Babesia bigemina, and Anaplasma marginale in water buffalo in Western Cuba. **Parasitology research**, 118:955–967.

Pearson K (1909) Determination of the coefficient of correlation. **Science**, 30:23–25.

Ribeiro MF, Lima JD (1995) Influence of temperature on the development of Anaplasma marginale in Boophilus microplus. **Arq. bras. med. vet. zootec**, 525–533.

Ribeiro MFB, Lima JD (1996) Morphology and development of Anaplasma marginale in midgut of engorged female ticks of Boophilus microplus. **Veterinary parasitology**, 61:31–39.

Ridder DA, Lang M-F, Salinin S, Röderer J-P, Struss M, Maser-Gluth C, Schwaninger

M (2011) TAK1 in brain endothelial cells mediates fever and lethargy. **Journal of Experimental Medicine**, 208:2615–2623.

Sankararaman S, Sridhar S, Kimmel G, Halperin E (2008) Estimating local ancestry in admixed populations. **The American Journal of Human Genetics**, 82:290–303.

Schwerin M, Czernek-Schäfer D, Goldammer T, Kata SR, Womack JE, Pareek R, Pareek C, Walawski K, Brunner RM (2003) Application of disease-associated differentially expressed genes–Mining for functional candidate genes for mastitis resistance in cattle. **Genetics Selection Evolution**, 35:1–16.

Sherman BT, Lempicki RA (2009) Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. **Nature protocols**, 4:44.

Shook GE, Schutz MM (1994) Selection on somatic cell score to improve resistance to mastitis in the United States. **Journal of Dairy Science**, 77:648–658.

Silva LOC, Gondo A, Nobre PRC, EUCLIDES FILHO K, Rosa AN, Josahkian LA, Figueiredo GR (2002) Genetic trends in Nellore breed in Brazil. In: Proc. 7th World Congress on Genetics Applied to Livestock Production, Montpellier. WCGALP, CD, **Anais**...

Team RC (2013) R: A language and environment for statistical computing.

Thwe PM, Pelgrom LR, Cooper R, Beauchamp S, Reisz JA, D'Alessandro A, Everts B, Amiel E (2017) Cell-intrinsic glycogen metabolism supports early glycolytic reprogramming required for dendritic cell immune responses. **Cell metabolism**, 26:558–567.

Tong Y, Lear TB, Evankovich J, Chen Y, Londino JD, Myerburg MM, Zhang Y, Popescu ID, McDyer JF, McVerry BJ (2020) The RNFT2/IL-3Rα axis regulates IL-3 signaling and innate immunity. **JCI insight**, 5:.

Utech KBW, Wharton RH, Kerr JD (1978) Resistance to Boophilus microplus (Canestrini) in different breeds of cattle. **Australian Journal of Agricultural Research**, 29:885–895.

VanRaden PM (2008) Efficient methods to compute genomic predictions. **Journal of dairy science**, 91:4414–4423.

Weber MM, Faris R, McLachlan J, Tellez A, Wright WU, Galvan G, Luo Z-Q, Samuel JE (2016) Modulation of the host transcriptome by Coxiella burnetii nuclear effector Cbu1314. **Microbes and infection**, 18:336–345.

Westwell-Roper C, Denroche HC, Ehses JA, Verchere CB (2016) Differential activation of innate immune pathways by distinct islet amyloid polypeptide (IAPP) aggregates. **Journal of Biological Chemistry**, 291:8908–8917.

Xue F, Li H, Zhang J, Lu J, Xia Y, Xia Q (2013) miR-31 regulates interleukin 2 and kinase suppressor of ras 2 during T cell activation. **Genes & Immunity**, 14:127–131.

Zeyen L, Döring T, Stieler JT, Prange R (2020) Hepatitis B subviral envelope particles

use the COPII machinery for intracellular transport via selective exploitation of Sec24A and Sec23B. **Cellular microbiology**, 22:e13181.

Zhao C, Liu H, Xiao T, Wang Z, Nie X, Li X, Qian P, Qin L, Han X, Zhang J (2019) CRISPR screening of porcine sgRNA library identified host factors essential for Japanese encephalitis virus replication. **bioRxiv**, 840835.

3.6. Supplementary material

Supplementary table 1. Genes positioned into a Windows of 1Mb to the top ten regions indicated by GWAS.

Gene ID	Chr	Windows (start-end)	Gene (start-end)	Gene Name	Description
ENSBTAG00000000379	5	88215591-89215591	88683333-88697403	*GOLT1B*	golgi transport 1B
ENSBTAG00000002023	17	57204948-58204948	57763560-57876534	*NOS1*	nitric oxide synthase 1
ENSBTAG00000002278	10	61002794-62002794	61653913-61919176	*FBN1*	fibrillin 1
ENSBTAG00000002551	5	88215591-89215591	88434139-88442014	*KCNJ8*	potassium inwardly rectifying channel subfamily J member 8
ENSBTAG00000002746	8	50939096-51939096	51153923-51214968	*OSTF1*	osteoclast stimulating factor 1
ENSBTAG00000003087	15	4490897-5490897	5477588-5506305	*DCUN1D5*	defective in cullin neddylation 1 domain containing 5
ENSBTAG00000003282	5	88215591-89215591	88834636-88889384	*SLCO1A2*	solute carrier organic anion transporter family member 1A2
ENSBTAG00000004011	10	61002794-62002794	60924617-61080535	*GALK2*	galactokinase 2
ENSBTAG00000004620	13	38320221-39320221	38404270-38419061	*ZNF133*	zinc finger protein 133
ENSBTAG00000004622	13	38320221-39320221	38428211-38533119	*DZANK1*	double zinc ribbon and ankyrin repeat domains 1
ENSBTAG00000005202	13	38320221-39320221	38706055-38706764	*SCP2D1*	SCP2 sterol binding domain containing 1
ENSBTAG00000005444	2	3744576-4744576	4300416-4399011	*UGGT1*	UDP-glucose glycoprotein glucosyltransferase 1
ENSBTAG00000005987	2	3744576-4744576	4676998-4785534	*WDR33*	WD repeat domain 33
ENSBTAG00000006003	2	3744576-4744576	4454874-4530094	*SAP130*	Sin3A associated protein 130
ENSBTAG00000007137	17	57204948-58204948	57183198-57231988	*RFC5*	replication factor C subunit 5
ENSBTAG00000007321	20	12501001-13501001	13439033-13484492	*SREK1*	splicing regulatory glutamic acid and lysine rich protein 1
ENSBTAG00000008089	2	3744576-4744576	4601012-4621042	*AMMECR1L*	AMMECR1 like
ENSBTAG00000008101	8	50939096-51939096	51911490-52419336	*PCSK5*	proprotein convertase subtilisin/kexin type 5
ENSBTAG00000008204	8	50939096-51939096	51058247-51098110	*CARNMT1*	carnosine N-methyltransferase 1
ENSBTAG00000008825	13	38320221-39320221	38533190-38546652	*POLR3F*	RNA polymerase III subunit F
ENSBTAG00000008826	13	38320221-39320221	38548013-38555741	*RBBP9*	"RB binding protein 9

ENSBTAG00000008964	13	38320221-39320221	38600324-38679626	*DTD1*	D-aminoacyl-tRNA deacylase 1
ENSBTAG00000010530	5	88215591-89215591	88796314-88801862	*IAPP*	islet amyloid polypeptide
ENSBTAG00000011661	10	61002794-62002794	61437230-61529573	*CEP152*	centrosomal protein 152
ENSBTAG00000011882	13	38320221-39320221	38891310-39323725	*SLC24A3*	solute carrier family 24 member 3
ENSBTAG00000013231	8	50939096-51939096	51044476-51050554	*C8H9orf40*	chromosome 8 C9orf40 homolog
ENSBTAG00000013333	5	88215591-89215591	88602173-88658602	*GYS2*	glycogen synthase 2
ENSBTAG00000013425	10	61002794-62002794	61080768-61113961	*COPS2*	COP9 signalosome subunit 2
ENSBTAG00000017260	5	88215591-89215591	89133571-89283293	*PDE3A*	phosphodiesterase 3A
ENSBTAG00000017602	29	35255483-36255483	36039644-36067845	*TMEM45B*	transmembrane protein 45B
ENSBTAG00000017610	29	35255483-36255483	36070018-36099972	*NFRKB*	nuclear factor related to kappaB binding protein
ENSBTAG00000018160	24	39324525-40324525	39951775-40062824	*LAMA1*	laminin subunit alpha 1
ENSBTAG00000018295	13	38320221-39320221	38561341-38592220	*SEC23B*	SEC23 homolog B"
ENSBTAG00000018978	13	38320221-39320221	38333657-38360021	*KAT14*	lysine acetyltransferase 14
ENSBTAG00000019001	29	35255483-36255483	36100991-36167328	*PRDM10*	PR/SET domain 10
ENSBTAG00000019036	24	39324525-40324525	39834619-39935989	*ARHGAP28*	Rho GTPase activating protein 28
ENSBTAG00000019294	5	88215591-89215591	88262950-88412938	*ABCC9*	ATP binding cassette subfamily C member 9
ENSBTAG00000019602	5	88215591-89215591	89019014-89099984	*SLCO1C1*	solute carrier organic anion transporter family member 1C1
ENSBTAG00000019603	5	88215591-89215591	88544523-88562783	*LDHB*	lactate dehydrogenase B
ENSBTAG00000019687	8	50939096-51939096	51126936-51149034	*NMRK1*	nicotinamide riboside kinase 1
ENSBTAG00000021078	5	88215591-89215591	88729876-88756498	*PYROXD1*	pyridine nucleotide-disulphide oxidoreductase domain 1
ENSBTAG00000021083	5	88215591-89215591	88697522-88730250	*RECQL*	RecQ like helicase
ENSBTAG00000022329	5	88215591-89215591	88901347-88970366	*SLCO1B3*	solute carrier organic anion transporter family member 1B3
ENSBTAG00000022583	10	61002794-62002794	61284100-61419065	*SHC4*	SHC adaptor protein 4
ENSBTAG00000025964	20	12501001-13501001	12444739-12621942	*MAST4*	microtubule associated serine/threonine kinase family member 4
ENSBTAG00000027764	10	61002794-62002794	61370363-61372119	*EID1*	EP300 interacting inhibitor of differentiation 1
ENSBTAG00000029126	5	88215591-89215591	89188741-89188850	*5S_rRNA*	5S ribosomal RNA
ENSBTAG00000032097	17	57204948-58204948	57900735-57938125	*FBXO21*	F-box protein 21

ENSBTAG00000032827	13	38320221-39320221	38680278-38680396	*5S_rRNA*	5S ribosomal RNA
ENSBTAG00000034827	15	4490897-5490897	4259082-4536080	*PDGFD*	platelet derived growth factor D
ENSBTAG00000036183	17	57204948-58204948	58026797-58141074	*FBXW8*	F-box and WD repeat domain containing 8
ENSBTAG00000042573	5	88215591-89215591	88954274-88954376	*U6*	U6 spliceosomal RNA
ENSBTAG00000043976	17	57204948-58204948	58188410-58250340	*RNFT2*	"ring finger protein
ENSBTAG00000044078	17	57204948-58204948	57955861-58020267	*TESC*	tescalcin
ENSBTAG00000044119	17	57204948-58204948	57231745-57664911	*KSR2*	kinase suppressor of ras 2
ENSBTAG00000044347	29	35255483-36255483	35919871-35919970	*U6*	U6 spliceosomal RNA
ENSBTAG00000046046	24	39324525-40324525	40126526-40129341	*LRRC30*	leucine rich repeat containing 30
ENSBTAG00000046553	10	61002794-62002794	61182241-61240007	*SECISBP2L*	SECIS binding protein 2 like
ENSBTAG00000048973	10	61002794-62002794	61825309-61825391	*bta-mir-10168*	bta-mir-10168
ENSBTAG00000049472	17	57204948-58204948	57335124-57335230	*U6*	U6 spliceosomal RNA
ENSBTAG00000049672	13	38320221-39320221	38988078-38988147	*bta-mir-11973*	bta-mir-11973
ENSBTAG00000050824	2	3744576-4744576	4187288-4227153	*HS6ST1*	heparan sulfate 6-O-sulfotransferase 1
ENSBTAG00000051249	13	38320221-39320221	38593653-38595044	*SMIM26*	small integral membrane protein 26
ENSBTAG00000051618	13	38320221-39320221	38329976-38333880	*PET117*	PET117 homolog
ENSBTAG00000053244	2	3744576-4744576	4623473-4636747	*POLR2D*	RNA polymerase II subunit D
ENSBTAG00000053951	5	88215591-89215591	88663823-88673433	*SPX*	spexin hormone

Supplementary table 2. List of quantitative trait loci (QTL) within 1Mb windows for each region indicated by GWAS.

Chr	Windows (start-end)	QTL (start-end)	QTL ID	PubMEd ID	QTL Trait
2	3744576-4744576	3977230-3977234	65418	26445451	Maturity rate
2	3744576-4744576	4200140-4200144	66852	19966163	Body weight (yearling)
2	3744576-4744576	4200140-4200144	66853	19966163	Body weight gain
2	3744576-4744576	4687704-4687708	135851	28619006	Tick resistance
2	3744576-4744576	4634987-4634991	36370	25149327	Meat color L*
5	88215591-89215591	89051204-89051208	65812	ISU0080	Sperm motility
5	88215591-89215591	89147993-89147997	66309	19966163	Body weight gain
5	88215591-89215591	89004548-89004552	67154	19966163	Body weight (yearling)
5	88215591-89215591	88364483-88364487	148995	29284405	Milk fat percentage
5	88215591-89215591	88271838-88271842	123389	27889128	Length of productive life
5	88215591-89215591	88412130-88412134	106942	27209127	Daughter pregnancy rate
5	88215591-89215591	88299090-88299094	102403	27287773	Milk yield
5	88215591-89215591	88285948-88285952	103295	27287773	Milk fat yield
5	88215591-89215591	88299090-88299094	103384	27287773	Milk protein yield
5	88215591-89215591	88271838-88271842	103415	27287773	Interval to first estrus after calving
5	88215591-89215591	88404779-88404783	121643	26193888	Calving interval
5	88215591-89215591	89051204-89051208	120257	27610941	Sperm motility
5	88215591-89215591	89004548-89004552	173408	31139206	Daughter pregnancy rate
5	88215591-89215591	88267555-88267559	173466	31139206	Milk fat percentage
5	88215591-89215591	88267555-88267559	174019	31139206	Milk fat yield
5	88215591-89215591	88921829-88921833	96244	26960806	Bovine tuberculosis susceptibility
5	88215591-89215591	88647265-88647269	174407	31139206	Milk yield
5	88215591-89215591	88432156-88432160	176717	31299913	First service conception
5	88215591-89215591	88287850-88287854	176360	31139206	Conception rate
5	88215591-89215591	88389453-88389457	166480	30459810	Milk fat yield
5	88215591-89215591	88432156-88432160	176993	31299913	Conception rate

5	88215591-89215591	88310753-88310757	41430	21831322	Calving ease (maternal)
5	88215591-89215591	88310753-88310757	41431	21831322	Calving ease
5	88215591-89215591	88310753-88310757	41432	21831322	Stillbirth
5	88215591-89215591	88332752-88332756	41434	21831322	Daughter pregnancy rate
5	88215591-89215591	88332752-88332756	41436	21831322	Somatic cell score
5	88215591-89215591	88332752-88332756	41438	21831322	Udder depth
5	88215591-89215591	88560626-88560630	20139	22281351	Milk fat percentage
5	88215591-89215591	88867416-88867420	41439	21831322	Body depth
5	88215591-89215591	88867416-88867420	41442	21831322	Foot angle
5	88215591-89215591	88867416-88867420	41443	21831322	Feet and leg conformation
5	88215591-89215591	88867416-88867420	41444	21831322	Milk fat percentage
5	88215591-89215591	88867416-88867420	41445	21831322	PTA type
5	88215591-89215591	88867416-88867420	41446	21831322	Teat placement - front
5	88215591-89215591	88867416-88867420	41447	21831322	Udder attachment
5	88215591-89215591	88867416-88867420	41448	21831322	Milk fat yield
5	88215591-89215591	88867416-88867420	41449	21831322	Net merit
5	88215591-89215591	88867416-88867420	41450	21831322	Length of productive life
5	88215591-89215591	88867416-88867420	41451	21831322	Milk protein percentage
5	88215591-89215591	88867416-88867420	41452	21831322	Milk protein yield
5	88215591-89215591	88867416-88867420	41453	21831322	Rear leg placement - rear view
5	88215591-89215591	88867416-88867420	41454	21831322	Rear leg placement - side view
5	88215591-89215591	88867416-88867420	41457	21831322	Stature
5	88215591-89215591	88867416-88867420	41458	21831322	Strength
5	88215591-89215591	88867416-88867420	41459	21831322	Teat length
5	88215591-89215591	88928018-88928022	30041	22100599	Interval to first estrus after calving
5	88215591-89215591	89004548-89004552	41462	21831322	Dairy form
5	88215591-89215591	89004548-89004552	41467	21831322	Udder height
5	88215591-89215591	89004548-89004552	41468	21831322	Rump width
5	88215591-89215591	89004548-89004552	41471	21831322	Udder cleft
5	88215591-89215591	89051204-89051208	57043	26198991	Heat tolerance
5	88215591-89215591	89124171-89124175	26177	22449276	Milk protein yield

8	50939096-51939096	50995574-50995578	67946	19966163	Body weight (yearling)
8	50939096-51939096	50995574-50995578	67947	19966163	Body weight gain
8	50939096-51939096	50995574-50995578	151469	29163638	Muscle anserine content
8	50939096-51939096	51225137-51225141	65971	26641032	Body weight
8	50939096-51939096	51274523-51274527	43657	21831322	Body depth
8	50939096-51939096	51274523-51274527	43658	21831322	Dairy form
8	50939096-51939096	51274523-51274527	43659	21831322	Feet and leg conformation
8	50939096-51939096	51274523-51274527	43660	21831322	PTA type
8	50939096-51939096	51274523-51274527	43661	21831322	Udder attachment
8	50939096-51939096	51274523-51274527	43662	21831322	Udder height
8	50939096-51939096	51274523-51274527	43663	21831322	Rump width
8	50939096-51939096	51274523-51274527	43664	21831322	Stature
8	50939096-51939096	51274523-51274527	43665	21831322	Udder cleft
8	50939096-51939096	51344990-51344994	43671	21831322	Rear leg placement - rear view
8	50939096-51939096	51344990-51344994	43672	21831322	Teat placement - rear
10	61002794-62002794	61879923-61879927	70034	25989905	Milk zinc content
10	61002794-62002794	61439592-61439596	96338	26960806	Bovine tuberculosis susceptibility
10	61002794-62002794	61462406-61462410	176422	31139206	Conception rate
10	61002794-62002794	61462406-61462410	157788	29705414	Milking speed
10	61002794-62002794	61804301-61804305	154154	29459679	Stature
10	61002794-62002794	61365125-61365129	65946	26641032	Pregnancy rate
13	38320221-39320221	38800333-38800337	118690	27485317	Milk unglycosylated kappa-casein percentage
13	38320221-39320221	38352320-38352324	152327	29163638	Polyunsaturated fatty acid content
13	38320221-39320221	38416336-38416340	151981	29163638	Muscle potassium content
13	38320221-39320221	38719392-38719396	181305	31718557	Conception rate
13	38320221-39320221	38550116-38550120	23833	23851991	Metabolic body weight
13	38320221-39320221	38955571-38955575	154811	27506634	Somatic cell count
13	38320221-39320221	39271474-39271478	23834	23851991	Residual feed intake
15	4490897-5490897	4692830-4692834	68768	19966163	Body weight (yearling)
15	4490897-5490897	5460596-5460600	69941	25989905	Milk potassium content
15	4490897-5490897	4913745-4913749	32419	25288516	Somatic cell score

15	4490897-5490897	5160954-5160958	32295	24909189	Milk conjugated linoleic acid content
17	57204948-58204948	57524993-57524997	126258	28193175	Milk palmitic acid content
17	57204948-58204948	57300081-57300085	163846	29391528	Milk caprylic acid content
17	57204948-58204948	57716041-57716045	152009	29163638	Muscle potassium content
17	57204948-58204948	57778571-57778575	160253	30229962	Bovine respiratory disease susceptibility
17	57204948-58204948	57286343-57286347	56545	24183684	Dry matter intake
17	57204948-58204948	57562660-57562664	16248	21198698	Milk fat yield
17	57204948-58204948	57562660-57562664	16249	21198698	Milk protein yield
17	57204948-58204948	57562660-57562664	16250	21198698	Milk fat percentage
17	57204948-58204948	57562660-57562664	16251	21198698	Milk protein percentage
17	57204948-58204948	57562660-57562664	16252	21198698	Interval from first to last insemination
20	12501001-13501001	12809253-12809257	65588	26445451	Maturity rate
20	12501001-13501001	13385887-13385891	109062	27485317	Milk casein percentage
20	12501001-13501001	12685470-12685474	177238	31299913	Conception rate
20	12501001-13501001	12528285-12528289	20466	22394233	Lean meat yield
20	12501001-13501001	12809253-12809257	36977	25273628	Lean meat yield
20	12501001-13501001	12932939-12932943	25652	22449276	Milk yield
20	12501001-13501001	13157395-13157399	20181	22281351	Milk protein percentage
24	39324525-40324525	39590561-39590565	138388	28219476	Twinning
24	39324525-40324525	39568134-39568138	151969	29163638	Muscle potassium content
24	39324525-40324525	39687341-39687345	181396	31718557	Conception rate
24	39324525-40324525	39714743-39714747	154972	27506634	Milk eicosapentaenoic acid content
24	39324525-40324525	39368913-39368917	24677	24906442	Marbling score
29	35255483-36255483	35951278-35951282	69455	19966163	Body weight gain
29	35255483-36255483	36092656-36092660	113121	27485317	Milk kappa-casein percentage
29	35255483-36255483	35511611-35511615	155240	27506634	Milk caproic acid content
29	35255483-36255483	35884891-35884895	152461	29163638	Tenderness score
29	35255483-36255483	35447078-35447082	30434	22100599	Interval to first estrus after calving
29	35255483-36255483	35447078-35447082	28167	24341352	Udder attachment
29	35255483-36255483	35697576-35697580	20350	22394233	Longissimus muscle area
29	35255483-36255483	35884891-35884895	35946	20630249	Milk protein percentage

29	35255483-36255483	36221963-36221967	26948	22449276	Milk protein percentage
29	35255483-36255483	35386091-35386095	181846	31931710	Abomasum displacement

CAPÍTULO 4 – Considerações Finais

Nossos resultados fornecem subsídio para compreensão das bases genéticas envolvidas na susceptibilidade de bovinos a infecções por *Babesia bigemina* (BigIL) e *Anaplasma margina* (AmIL). A incorporação de BigIL em modelos de predição de AmIL ou vice-versa melhoram a capacidade preditiva das características pela existência de correlação genética entre ambas. Embora o carrapato *Rhipicephalus microplus* seja o principal vetor da babesiose e anaplasmose bovina, não identificamos conexão entre contagem de carrapatos e parasitemia, sendo dispensável a informação de contagem de carrapatos em modelos de predição das infecções causadas pela tristeza parasitária bovina.