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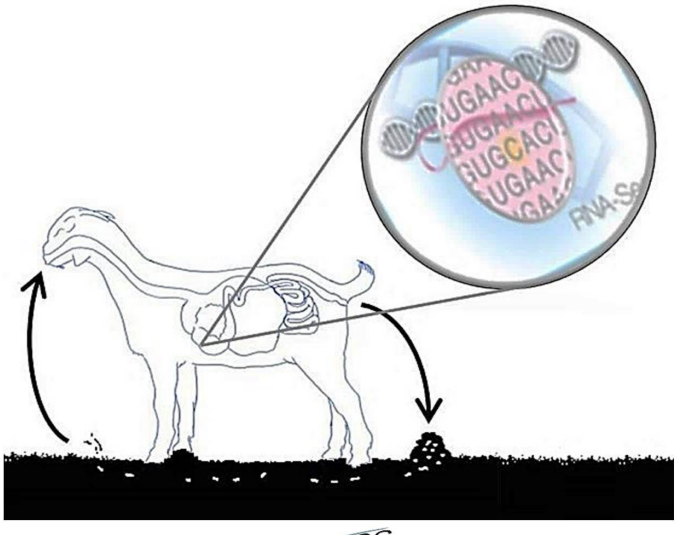

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Abstract

Gastrointestinal nematode (GIN) infections are one of the major constrains for sheep and goat production worldwide. One of the promising control strategies is the genetic selection for resistant animals as there are no issues due to anthelmintic resistance and it aligns to demands for chemical-free food. Exploring possible phenotypic and genomic markers that could be used in breeding scheme besides understanding the mechanisms responsible for resistance were the main goals of this thesis.

Thesis consists of **General introduction**, a brief description of GIN biology and methods to control GIN with focus on phenotypic and genomic markers, **four papers** and **General discussion**. In **paper I**, a systematic review and meta-analysis were conducted to re-analyse and summarize the findings on immunoglobulins response to GIN in the literature and discuss the potential to use immunoglobulins as biomarkers of the host resistance. A conceptual model summarizing the role of immunoglobulins in resistance to GIN is proposed. In **paper II**, transcriptome profiling of the abomasal mucosa and lymph node tissues were compared between non-infected, resistant and susceptible Creole goats experimentally infected with *Haemonchus contortus*. Results indicated that the maintenance of the integrity of the mucosa has probably the priority for the host at late infection stage. In **paper III**, the dynamics of the response of the abomasal mucosa of resistant and susceptible Creole goats experimentally infected with *H. contortus* were compared. The immune response was activated through many relevant pathways including the Th1 immune response at different time post-infection. Interestingly, the results showed a simultaneous time series activation of Th2 related genes in resistant compared to susceptible kids. In **paper IV**, the genomic variants of Creole goats resistant and susceptible to *H. contortus* were discovered from RNA-sequencing data at four different times post-infection. Single nucleotide polymorphisms, insertions and deletions that distinguish the resistant and the susceptible groups were identified and characterized through functional analysis. The T cell receptor signalling pathway was one of the top significant pathways that distinguish the resistant from the susceptible group with genomic variants in 78% of genes in this pathway.

Keywords: small ruminants, *Haemonchus contortus*, genetic resistance, immune response, transcriptome

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Variations génomiques et mécanismes moléculaires de la réponse de l'hôte aux nématodes gastro-intestinaux chez les petits ruminants

Résumé

Les infections par les nématodes gastro-intestinaux (NGI) constituent l'une des contraintes principales de production chez les ovins et les caprins dans le monde. Une des solutions prometteuse est la sélection génétique d'animaux résistants aux NGI. Cette sélection permettrait de réduire l'utilisation des anthelminthiques et donc l'apparition de souches de NGI résistants à ces molécules, et de répondre à la demande sociétale de produits animaux sans résidus. Les principaux objectifs de cette thèse étaient de caractériser des marqueurs phénotypiques et génomiques qui pourraient être utilisés dans des schémas de sélection et de comprendre les mécanismes physiologiques sous-jacents. Ce manuscrit de thèse est structuré de la manière suivante : une introduction générale suivie d'une brève description de la biologie des NGI et des méthodes de contrôle en mettant l'accent sur les marqueurs phénotypiques et génomiques, puis les quatre articles scientifiques et la discussion générale. Dans l'article I, une revue systématique et une méta-analyse ont été réalisées pour ré-analyser et résumer les résultats de la littérature sur la réponse humorale contre les NGI et discuter de la possibilité d'utiliser les immunoglobulines comme biomarqueurs de la résistance de l'hôte. Un modèle conceptuel résumant le rôle des immunoglobulines dans la résistance au NGI est proposée. Dans l'article II, les transcriptomes de la muqueuse abomasale et des ganglions drainants d'animaux résistants et sensibles infestés par *Haemonchus contortus* et non-infestés ont été comparés. Les résultats ont montré qu'à un stade tardif de l'infestation le maintien de l'intégrité de la muqueuse est probablement la priorité pour l'hôte. Dans l'article III, les dynamiques de la réponse de l'hôte au niveau de la muqueuse abomasale ont été comparées entre animaux résistants et sensibles infestés par *H. contortus*. Nous avons montré l'activation de nombreuses voies de signalisation, notamment la voies Th1 et Th2 de manière concomitante. Dans l'article IV, des variants génomiques (SNP, insertions et délétions) de la résistance aux NGI ont été mis en évidence à partir des données de séquençage des ARN et caractérisés par une analyse fonctionnelle. L'une des voies de signalisation qui distinguent le mieux les deux génotypes est celle des récepteurs de lymphocytes T, près de 78% des gènes de cette voie de signalisation présentent des variants génomiques.

Mots-clés: petits ruminants, *Haemonchus contortus*, résistance génétique, réponse immunitaire, transcriptome

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Genomisk variation och molekylära mekanismer för värdens svar på gastrointestinala nematoder hos små idisslare

Sammanfattning

Infektioner med gastrointestinala nematoder (GIN) är en av de största begränsningarna för får- och getproduktion över hela världen. En av de lovande kontrollstrategierna är avel för resistenta djur eftersom det då inte finns några problem på grund av anthelmintisk resistens och det stämmer bra överens med efterfrågan på kemikaliefri mat. Huvudmålet för denna avhandling var att undersöka möjliga fenotypiska och genomiska markörer som kan användas i avelsprogram samt att förstå de mekanismer som är ansvariga för resistens.

Avhandlingen består av allmän introduktion, en kort beskrivning av biologin för GIN och metoder för att kontrollera GIN med fokus på fenotypiska och genomiska markörer, fyra artiklar och allmän diskussion. I artikel I genomfördes en systematisk granskning och metaanalys för att analysera och sammanfatta resultaten i litteraturen om immunoglobuliners svar på GIN och diskutera potentialen att använda immunoglobuliner som biomarkörer för resistens hos värden. En konceptuell modell som sammanfattar immunoglobuliners roll i resistens mot GIN föreslås. I artikel II jämfördes transkriptomprofilering av bukslemhinnan och lymfkörtelvävnader mellan icke-infekterade, resistenta och mottagliga kreolska getter som experimentellt infekterats med *Haemonchus contortus*. Resultaten indikerade att upprätthållandet av slemhinnans integritet troligen har prioritet för värden vid sen infektion. I artikel III jämfördes dynamiken i responsen hos bukslemhinnan hos resistenta och mottagliga kreolska getter som experimentellt infekterats med *H. contortus*. Immunsvaret aktiverades genom många relevanta reaktionsvägar inklusive Th1-immunsvaret vid olika tidpunkter efter infektion. Intressant nog visade resultaten en samtidig aktivering av Th2-relaterade gener hos resistenta jämfört med mottagliga kid. I artikel IV upptäcktes de genomiska varianterna hos kreolska getter som var resistenta och mottagliga för *H. contortus* från RNA-sekvenseringsdata vid fyra olika tidpunkter efter infektion. Variation i enstaka baspar, insertioner och deletioner som skiljer de resistenta och mottagliga grupperna identifierades och karakteriserades genom funktionell analys. T-cellreceptorsignalvägen var en av de viktigaste reaktionsvägarna som skiljer den resistenta från den mottagliga gruppen med genomiska varianter i 78% av generna i denna reaktionsväg.

Nyckelord: små idisslare*, Haemonchus contortus,* genetisk resistens*,* immunsvar, transkiptom

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Dedication

To my loving, supportive, encouraging, and patient husband Mohamed Rashid, none of this would have been possible without your love and support.

To my family in Egypt for all their love and encouragement.

Thank you!!

Contents

List of publications

This thesis is based on the work contained in the following papers:

I Aboshady H. M., M.J. Stear, A. M. Johansson, E. Jonas, J.C. Bambou* (2019). Immunoglobulins as Biomarkers for Gastrointestinal Nematodes Resistance in Small Ruminants. (submitted)

II Aboshady H. M., N. Mandonnet, M. J. Stear, R. Arquet, M. Bederina, J. Sarry, G. Tosser-Klopp, C. Klopp, A. M. Johansson, E. Jonas, J.C. Bambou* (2019). Transcriptome variation in response to gastrointestinal nematode infection in goats. *PLoS ONE* 14(6): e0218719. *https://doi.org/10.1371/journal.pone.0218719*

III Aboshady H. M.; N. Mandonnet; Y. Félicité; J. Hira; A. Fourcot; C. Barbier; A. M. Johansson; E. Jonas; J.C. Bambou* (2019). Dynamic transcriptomic changes of goat abomasal mucosa in response to *Haemonchus contortus* infection. (submitted)

IV Aboshady H. M., N. Mandonnet, A. M. Johansson, E. Jonas, J.C. Bambou*. Genomic variants from RNA-seq for goats resistant or susceptible to gastrointestinal nematode infection. (submitted)

* Corresponding author.

The contribution of Aboshady H. M. to the papers included in this thesis was as follows:

I Performed the literature search, the statistical analysis and contributed to interpretation of the data and to writing of the manuscript.

II Formal analysis and writing – original draft.

III Performed bioinformatics and statistical analysis, interpretation of the data and writing – original draft.

IV Performed bioinformatics and statistical analysis, methodology, interpretation of the data and writing – original draft.

Abbreviations

C	Chitinases
CarLA	saliva IgA antibody
CCR4	C-C Motif Chemokine Receptor 4
Chi3L1	chitinase-3 like 1
Chi3L2	chitinase-3 like 2
CLP	chitinase-like proteins
DEG	differentially expressed genes
dpi	days post infection
DUOX	dual oxidase
EBV	estimated Breeding Value
EOS	eosinophils
FEC	faecal egg count
GATA3	GATA binding protein 3
GIN	gastrointestinal nematode
GWAS	genome-wide association study
IFN-γ	interferon ɣ
Ig	immunoglobulin
IL	interleukin
IL17F	interleukin 17F
IL2RG	interleukin 2 receptor subunit gamma
IL4R	interleukin 4 receptor
L1	first stage larvae
L2	second stage larvae
L3	third stage larvae
L4	fourth stage larvae
L5	Immature adults
MHC	major Histocompatibility Complex
NOS2A	nitric oxide synthase
PCV	packed cell volume

PHOX	phagocytic oxidase
QTL	quantitative trait loci
rHc23	recombinant form of *H. contortus* somatic antigen
RNA-seq	RNA sequencing
RONS	reactive oxygen and nitrogen species
RORC	RAR Related Orphan Receptor C
SNP	single nucleotide polymorphism
STAT3	Signal transducer and activator of transcription 3
STAT6	Signal transducer and activator of transcription 6
TCR	T cell receptor
TGF-β	transforming growth factor-β
Th	T helper
TNF-β	tumor necrosis factor β
Tregs	regulatory T cells

1 General introduction

1.1 Global context

The world population is predicted to grow by over one third between 2009 and 2050 reaching expectably 9.8 billion in 2050 while it is expected to surpass 11.2 billion in 2100 according to official estimations from the united nation in 2017 (https://refugeesmigrants.un.org/es/node/100043622#collapseOne). The population is expected to increase rapidly in developing country in Africa and Asia, while the population in developed countries is expected to increase slightly.

This population growth leads to the challenge for agriculture to produce more food to feed a growing population and to adopt more efficient and sustainable production methods. Ruminant in general and small ruminants in particular are known for their ability to eat low valuable resources (low inputs) to produce high valuable products (increase outputs). Small ruminants have a very valuable contribution in production of goods for human needs throughout the world, ranging from food with precious animal proteins (meat and milk) to fibre and skins, draught power in the highlands, food security and important non-market services. Additionally, small ruminants make important contributions to human livelihoods in small farming systems and developing economies. Recent reports from the Food and Agriculture Organization (http://www.fao.org) showed that Asia counts for 37 and Africa for 22% of the 1.2 billion world sheep population together with 56 and 30% of the approximately 1 billion world goat population, respectively.

Goats, in particular, are known for their ability to survive in some of the most inhospitable regions of the world and are usually called the ‘poor man’s cow’ which underlines their importance in small farming systems. Recent

reports from the FAO showed that goat population is expanding and more than 95% of the population is found in developing countries.

1.2 Gastrointestinal nematode in small ruminants production

One of the main wedges for efficient livestock farming is management of animal health. Among the diseases that constrain the productivity of sheep and goats, gastrointestinal nematode (GIN) infection ranks highest on a global index. GIN parasite affects productive and reproductive performance and leads to economic losses (Mavrot, Hertzberg and Torgerson, 2015). Among all the species of GIN commonly found in small ruminants, *Haemonchus contortus*, *Trichostrongylus spp*. and *Teladorsagia circumcincta* are the most abundant and cause the greatest losses in production. *H. contortus* is known to be the most important nematode species of small ruminants in tropical and subtropical areas, meanwhile in temperate regions, the most economically important nematodes are *Trichostrongylus spp*. and *T. circumcincta* (Peter and Chandrawathani, 2005; O'Connor, Walkden-Brown and Kahn, 2006). However, it has been reported an increasingly common occurrence of *H. contortus* also in temperate areas such as in Sweden, France, Denmark and the Netherlands (Waller *et al.*, 2004). This phenomenon is expected to aggravate with accordance to expected increase in temperature worldwide and climate changes reported in the last IPCC (Intergovernmental Panel on Climate Change) report in 2019.

Generally, GIN have a simple direct life cycle presented in figure 1. The development of the nematode larvae has five stages within two phases: the free-living phase in the external environment and the parasitic phase. The free-living phase starts from eggs which are dispersed on the pasture by animal faeces. The eggs hatch thereafter into first stage larvae (L1) and develop to the second (L2) and third (L3) larval stage. L3 is a non-feeding stage which could last for weeks to months depending on the environmental condition. The parasitic phase starts after the host ingests forage containing infective larvae (L3). These L3 lose their sheath in the host to become parasitic L3 and migrate to their host organ (abomasum or small intestinal). The larvae enter the gastric glands where they have their third molt and develop into the fourth larval stage (L4), as which they move then back into the lumen of the gastrointestinal tract. After another molting, the L4 develop into immature adults (L5) for a short period of time before becoming mature adults (Soulsby, 1982).

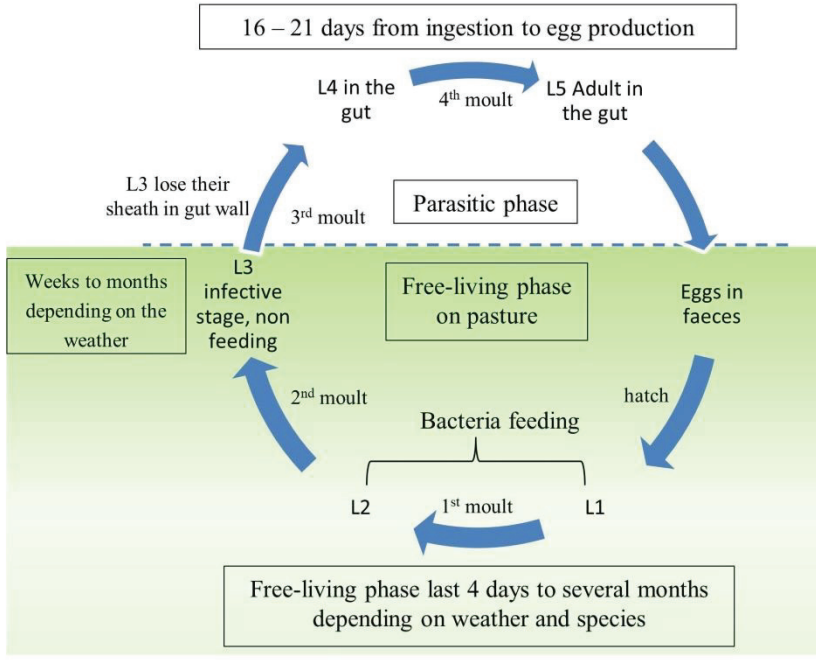

Figure 1. Gastrointestinal nematode life cycle.

1.3 Non-genetic methods to control GIN

There are different approaches to control GIN infection, each of them either target the parasite population in the host or on pasture, but all of them have the same goal which is minimize the impact of GIN on animal performance by minimizing host parasite contact (Jackson and Miller, 2006). The main methods that can be used to control GIN include chemical/anthelmintic methods, grazing management, nutrition, biological or vaccines.

Chemical control is the most widely used method for control GIN infection. The rapid and broad advance of anthelmintic drugs in the early 1960s offered an affordable and simple way to manage GINs. As a result, these drugs have been widely used as a cost-effective means for GIN control. The frequencies use of anthelmintic leads to pathogen resistance. Anthelmintics resistance have been reported all over the world (Kaplan, 2004; Jabbar *et al.*, 2006; Falzon *et al.*, 2014; Zvinorova *et al.*, 2016). *H. contortus* is prominent amongst the reports of anthelmintic resistance that has emerged (Peter and Chandrawathani, 2005). Growing anthelmintic resistance has created a compelling need to develop alternative options for the control of GIN infection.

Grazing management has been utilized for many years as a mean of parasite control to limit the host-parasite contact hence reducing pasture contamination. The different strategies can be considered as being either preventative, evasive, or diluting. The preventative strategy involves turning out parasite-free animals on clean pastures such as delayed turn-out, change of pastures between seasons, and the use of more aftermath. The evasive strategy involves moving animals from contaminated to clean pastures such as changing the pasture within the same season. The diluting strategy allows diluting pasture infectivity by mixed or alternate grazing with other host species (Cabaret, Bouilhol and Mage, 2002). However, these grazing methods are difficult to apply in extensive production systems and in those with common grazing, besides in many intensive systems there may not be sufficient land for grazing, or adequate numbers of non- susceptible animals, to provide a sufficient reduction in the numbers of GIN on pasture (Jackson and Miller, 2006).

Some plants that showed bioactive effects on internal parasite populations may help on controlling GIN by either acting directly upon the parasite population and/or indirectly by influencing host mediated regulatory mechanisms (Jackson and Miller, 2006). Consequently, optimized animal nutrition could play a role in controlling GIN infection. A first report for the possible use of tanniferous plants to control different worm species was reported in New Zealand (Niezen *et al.*, 1998). The authors showed that some condensed tannins plants (*Hedysarium coronarium*) were able to reduce parasite burdens while other condensed tannins plants (*Lotus pedunculatus*) maintain animal performance despite high worm burden. In this context, a highly rich source of condensed tannin (quebracho extracts) supplementation induced reduction in *H. contortus* fecundity and faecal egg counts (FEC) in goats (Paolini *et al.*, 2003). However, the same condensed tannin extracts have been found to reduce small intestine burdens (*Trichostrongylus colubriformis, Cooperia, Nematodirus* and *Bunostomum spp*) but not those from the abomasum (*H. contortus* and *T. circumcinta*) in sheep (Athanasiadou *et al.*, 2001). There is also extensive evidence in several breeds of sheep indicating the benefits of improving nutrition through supplementation of dietary protein as a mean of parasite control (Steel, 2003).

A biological control method through nematode-trapping fungi (*Duddingtonia flagrans*) has been used in small ruminants for parasite control (Larsen *et al.*, 1997). Resting spores of this fungus break the lifecycle of parasites bypass through the digestive tract, deposit in the faeces and develop along with the larvae then trapping and killing the larvae before they migrate to pasture (Terrill *et al.*, 2012). Work with nematode trapping fungi was discontinued because of lack of a commercial source of the spores.

Internal parasites can be controlled by the use of vaccines. The general approach for identifying candidate vaccine antigens is to screen for a protective fraction against target parasite through preliminary protection trials, then to purify the protective fraction, isolate and express the genes which encode this protein. Finally, a functional recombinant protein can be produced (Jackson and Miller, 2006). For example, some vaccines derived from the worm's intestinal gut cells. Consequently when the parasite feeds on the host, the parasite ingests antibodies that bind to functional proteins on its intestinal surface. As a result, digestive processes are compromised leading to starvation, loss of fecundity, weakness and at the end parasites lost from the infected site (Jackson and Miller, 2006; Terrill *et al.*, 2012). Recently, a recombinant form of *H. contortus* somatic antigen (rHc23) have been produced and used successfully for vaccination against GIN (González-Sánchez, Cuquerella and Alunda, 2018). The problem associated with the use of vaccines could be related to the cost, the need for regular re-vaccination and that continued exposure to larval antigens can stimulate a natural immunity (Jackson and Miller, 2006; Zvinorova *et al.*, 2016).

1.4 Genetic control of GIN

Animals can combat the adverse effects of parasites with two broad strategies: resistance and tolerance. Resistance is defined as the host ability to reduce the probability of infection, reduce the growth of the pathogen population within it, or recover from infection. Tolerance, by contrast, is defined, as the ability to limit the damage caused by a given parasite burden and maintaining health, performance and ultimately on fitness as infections levels increase (Kause, 2011). Resilience is related to tolerance, and describes an animal's ability to maintain performance in the face of a disease challenge (Råberg, Sim and Read, 2007; Bishop, 2012). Different between resistance and tolerance are shown in figure 2.

Figure 2 shows that by selecting for low FEC or parasite load we select for resistant animals that could differ in tolerance level (more tolerant has lower slope). Meanwhile, animals with high FEC or parasite load are susceptible but also some of them more tolerant (with low slope). Other tolerant animals are lost as they have medium infection load. This explains the complicity in defining resistant and tolerant individuals in disease respect. Another point is that in a breeding program we give more weight for production level.

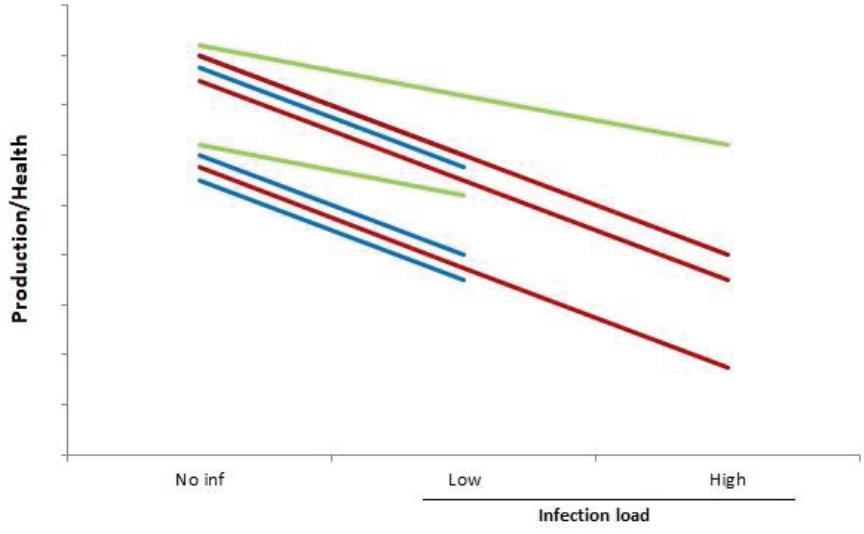

Figure 2. Schematic figure showing changes in productivity/health for different host genotypes (blue, red and green line) after exposed to same infection dose. The blue genotype has lower parasite burdens (is resistant). The red genotype has higher parasite burdens (is susceptible), red and blue equal in tolerance (same slope). The green genotype has lower slope (is tolerant), thereby maintains production/health status.

The term disease resistance is often used loosely and generically to cover both resistance to infection as well as resistance to the disease consequences of infection, that is, disease tolerance. In general, the susceptibility to nematode infections seems to be related to genetic factors since evidence for genetic variation in resistance to nematode infection has been observed within and between breed (Sayers and Sweeney, 2005) which make opportunity to the use of genetic variation in resistance for the purpose of breeding animals for increased GIN resistance. Moreover, genetic variation in tolerance has been recorded as genetic variance in regression slopes of host performance along a gradient of increasing pathogen burden (Kause, 2011).

1.4.1 Classical selection approach, phenotypic markers to GIN

Appropriate phenotypes traits that could be considered as indicator for resistance to nematodes have been classified (Bishop, 2012; Coutinho *et al.*, 2015) as follows:

- Measures of resistance: FEC, worm burden, worm size and fecundity.
- Immune response: Eosinophilia, antibodies such as IgA, IgG and IgM.
- Measures of impact of infection: anemia, pepsinogen or fructose amine concentrations.

- Measures of resilience: growth rate, anemia and/or required treatment frequency in relation with FEC, worm burden, worm size or fecundity.

Of these traits, FEC and anemia are the most studied traits. In animals infected with *H. contortus*, anemia can be easily measured using either PCV or the Famacha score, which is an indicator of anemia in the eyelid (Bishop, 2012).

Genetic variation in resistance to GIN within and between breeds has been studied extensively in sheep and goats as reviewed by Zvinorova *et al.* (Zvinorova *et al.*, 2016). Successful selection for nematode resistance has been reported in sheep and goats (Vagenas *et al.*, 2002). Conventional breeding strategies are based on the use of indicator traits to select for resistance. FEC have been the main indicator for resistance to GIN. FEC has been found to be a low to high heritable trait in lambs within the heritability range from 0.01 to 0.65, which is sufficiently high in most breeds to make selective breeding feasible (Stear, Strain and Bishop, 1999; Bishop, 2012; Zvinorova *et al.*, 2016). Moderate heritability for FEC was found in kids ranging from 0.1 to 0.37 (Mandonnet *et al.*, 2001; Vagenas *et al.*, 2002), which makes it still possible to breed for improved resistance to nematodes in goats. In addition, the differences in the estimated FEC heritability may be related to the age of animals as it has been reported (Stear, Strain and Bishop, 1999) that the heritability of a single egg count in each month of lambs age (Scottish Blackface) was essentially zero at 1 and 2 months of age, then rose rapidly to 0.33 at 6 months of age. Moreover, genetic correlations between FEC and resistance to different species of nematodes tend to be related being close to 0.5 (Bishop *et al.*, 2004).

Other traits that could be used to breed for improved resistance to nematodes are packed cell volume (PCV) (Mandonnet *et al.*, 2001; Baker *et al.*, 2003; Coutinho *et al.*, 2015), blood eosinophils (EOS) (Dawkins, Windon and Eagleson, 1989; Stear *et al.*, 2002), worm size and number of eggs in utero in adult female worms which are strongly heritable traits (Stear *et al.*, 1995, 1997). Meanwhile, the numbers of larvae or adult worms present in the gut are weakly inherited (Stear *et al.*, 1997).

Another heritable trait that could be used in traditional breeding as indicator trait for resistance to different nematodes species is antibody responses and it has been found to be moderately to strongly heritable. For example, Smith et al. (1985) were the first to show strong correlation of 0.95 between increased lymphatic IgA concentrations and reduced mean worm length, in 4.5 and 10 month-old lambs. Despite that there is no review available to evaluate the possibility of using immunoglobulins as phenotypic biomarkers in breeding schemes.

Although selection for resistance is possible and effective for sheep and goats, there are other issues restricting it. The main problem with conventional breeding strategies is the indicator traits which are costly, time consuming to collect and the need to infect animals (Zvinorova *et al.*, 2016).

1.4.2 Molecular genetic markers associated with GIN resistance

Incorporation of genotype information, using genetic markers approach, focuses on identifying DNA markers, which may not necessarily be causative mutations for resistance themselves, but may be in linkage disequilibrium with the causative mutation (Sayers and Sweeney, 2005). In contrast to the classical selection, marker-assisted selection can be utilized to accelerate selection with more efficiency even in cases where the desirable alleles for the trait are found in low frequencies, beside avoiding the requirement for animals to be challenged with nematodes (Bishop, 2012; Zvinorova *et al.*, 2016). A summary for previous studies that examined different molecular genetic marker association with GIN resistance is presented in appendix 1.

Associations with candidate-genes or specific markers

Several studies examined the association of specific genes or markers with FEC. In searching for genes involved in resistance or susceptibility, the genetic markers that have been most frequently associated with nematode resistant are those from the major histocompatibility complex (MHC) region on *Ovis aries* chromosome 20 (Schwaiger *et al.*, 1995; Buitkamp *et al.*, 1996; Janßen *et al.*, 2002; Sayers, Good, Hanrahan, Ryan, Angles, *et al.*, 2005; Davies *et al.*, 2006; Valilou *et al.*, 2015). Genes of this complex play important roles in presenting antigens to host T lymphocytes, causing T cell activation (Zinkernagel and Doherty, 1979). MHC genes were reported to have high levels of polymorphism (Schwaiger *et al.*, 1995; Valilou *et al.*, 2015). In this context, Bolormaa *et al.* (2010) tested specific markers on goat chromosome 23 which is near to the MHC region and found it to be associated with goat resistance to nematodes. The second most frequently identified gene in studies for resistance to GIN infection is the interferon ɣ (*IFN-γ*) gene on *O. aries* chromosome 3 (Coltman *et al.*, 2001; Sayers, Good, Hanrahan, Ryan and Sweeney, 2005). *IFN-γ* is known to be one of the principal cytokines produced by Th1 cells as innate immune response resulting in a cell mediated immune response (Schallig, 2000). The role of MHC and *IFN-γ* genes in immune response and their association with resistance and/or susceptibility to GIN infection are discussed in detail later in discussion section.

A main obstacle with candidate-genes or specific markers studies is that it is relied on prior knowledge to predict the correct genes or markers, usually on the basis of biological hypotheses or the location of the gene or marker within a previously determined region (Hirschhorn and Daly, 2005). However, lots of genes have their functions yet to be defined.

Microsatellite-based QTL studies

Quantitative trait loci (QTL) mapping can help in understanding the complexity of parasite resistance by identifying candidate genomic regions. Studies using microsatellite markers have been conducted to identify genomic region associated with GIN resistance. Several microsatellite-based QTL on different chromosomes have been reported in the literature for sheep. Most reported genomic regions for nematode resistance in sheep are located in chromosome 1, 3, 6, 14 and 20 (Davies *et al.*, 2006; Gutiérrez-Gil *et al.*, 2009; Stear *et al.*, 2009; Dominik *et al.*, 2010; Matika *et al.*, 2011; Silva *et al.*, 2011). Genomic regions on chromosome 2 were also reported for nematode resistance in sheep in many studies (Crawford *et al.*, 2006; Davies *et al.*, 2006; Marshall *et al.*, 2012; Sallé *et al.*, 2012). In a few studies, some other potential genomic regions were identified on different ovine chromosomes. It should be also noticeable that some studies used microsatellites that only cover 8 or 9 chromosomes and not the whole genome (Crawford *et al.*, 2006; Davies *et al.*, 2006; Dominik *et al.*, 2010). Meanwhile in goats, the first genome scan was undertaken in goats of the Creole breed and identified 13 QTL for resistance, resilience and immune criteria (de la Chevrotière *et al.*, 2012). The main conclusion from microsatellite-based QTL studies is that most significant QTL effects tend to be scattered throughout the genome.

Results from microsatellite-based QTL studies are often difficult to utilize in breeding programs, primary because the QTL are generally detected within families, and the markers linkage with causative mutation is family specific (within-family linkage). This explains why previously identified QTL seem to disappear with new ones emerging between populations (Bishop, 2012; Zvinorova *et al.*, 2016)..

SNP studies

An alternative to microsatellite-based QTL is the single nucleotide polymorphism (SNP) associations, in which SNPs are associated with favorable phenotypes across an entire population. This technique uses SNPs that show population-wide linkage disequilibrium with the causative mutation, consequently the issue of family-specific linkage is avoided (Bishop, 2012).

The availability of SNP arrays such as the GoatSNP50k chip, the OvineSNP50k chip and OvineSNP600k chip made Genome-wide association study (GWAS) more prevalence. GWAS aim at understanding the genetic basis of complex traits, such as resistance to diseases and production traits by searching the whole genome for genetic variants associated with the studied trait, without prior assumptions (Hirschhorn and Daly, 2005).

Results from GWAS reported genomic regions for nematode resistance in sheep on chromosomes 6 (Riggio *et al.*, 2013, 2014; Benavides *et al.*, 2015) and 14 (Riggio *et al.*, 2013, 2014), both regions were previously reported in microsatellite-based QTL studies. Meanwhile, other genomic region identified in many QTL studies were not reported using GWAS. Regions on sheep chromosome 4 (Riggio *et al.*, 2014), 7 (Benavides *et al.*, 2015) and 19 (Riggio *et al.*, 2014) were identified in GWAS. The only GWAS for nematode resistance in goats was in Creole goat (Silva *et al.*, 2018). Results from this study identified a total of seven SNP (on the chromosomes 4, 6, 11, and 17) associated with nematode resistance and the identified genes near to these positions were related to the intestine damage, inflammation process, immune response, hemorrhage control, and muscle weakness.

Evidence from SNP association studies suggests that individual SNPs are likely to be associated with very small effects because of polygenic nature of the resistance trait (Kemper *et al.*, 2011). As a result, to achieve reasonable genetic progress many SNPs would need to be included in a breeding program (Bishop, 2012). Moreover, obtaining GWAS for parasite resistance requires genotyping and phenotyping large numbers of animals (McCarthy *et al.*, 2008).

1.4.3 Genome- wide expression studies

A detailed understanding of the genes and biological mechanisms involved in resistance and protective immunity will aid the development of direct genetic markers which consider sustainable nematode control methods (McRae *et al.*, 2015). Gene expression profiling or transcriptional profiling allows examining large numbers of transcripts simultaneously in order to identify those transcripts that contribute to an animal's susceptibility or resistance.

The first studies that described genome-wide gene expression differences in parasite-resistant and susceptible sheep used the cDNA microarray technology (Diez-Tascón *et al.*, 2005; Keane *et al.*, 2006, 2007; Rowe *et al.*, 2008; MacKinnon *et al.*, 2009; Andronicos, Hunt and Windon, 2010; Knight *et al.*, 2011). Microarray technology is a tool to address complex biological questions by measurement and analysis gene expression simultaneously from potentially thousands of genes (Diez-Tascón *et al.*, 2005). Studying differentially

expressed genes (DEG) via microarray has led to the identification of genes and biological processes involved in the development of a resistant phenotype. Out of the identified genes, biological processes and pathways; genes involved in the stress and/or immune response were the most common (Diez-Tascón *et al.*, 2005; Keane *et al.*, 2006, 2007; Rowe *et al.*, 2008; MacKinnon *et al.*, 2009; Andronicos, Hunt and Windon, 2010; Knight *et al.*, 2011). In microarrays, samples of RNA populations are hybridized with DNA spots to determine the extent of expression of each sequence. As a result microarray technology has inherent weaknesses in terms of repeatability and precision because it relies on hybridization (’t Hoen *et al.*, 2008).

Instead of testing the expression of thousands of genes through microarray, nowadays RNA sequencing (RNA-seq) provides a tool for analysing the entire transcriptome of an organism. Identifying DEG through whole transcriptome analysis via RNA-seq and functional analysis for these genes has been shown to provide a key role in the knowledge of mechanisms responsible for complex quantitative traits (Costa *et al.*, 2013). Whole transcriptome analysis via RNA-seq have been used recently to identify DEG in resistance and susceptible sheep to GIN infection (Gossner *et al.*, 2013; Ahmed *et al.*, 2015; Guo *et al.*, 2016; McRae *et al.*, 2016). Meanwhile, only one study in goats used RNA-seq technology to explore the genetic resistance to GIN infection (Bhuiyan *et al.*, 2017). Identified DEG via RNA-seq from sheep and goats studies were involved mainly in inflammatory and immune responses.

Through RNA-seq, besides allowing the detection of DEG, functional genes are sequenced at high coverage, allowing to full scale variants discovery in coding genes. This technique has been used as a method to detect SNPs in transcribed regions in an efficient and cost-effective way for different traits and species (Cánovas *et al.*, 2010; Sharma *et al.*, 2012; Wang *et al.*, 2015; Martínez-Montes *et al.*, 2017; Pareek *et al.*, 2017). Up to date, there is no study explored genomic variants via RNA-seq related to resistance to GIN in sheep or goats.

Generally, studies in genetic resistance to nematode strongly suggest that the genetic resistance to GIN in small ruminants is closely linked to the host immune response. However, it appears that the underlying mechanisms are different at least partly, from breed to breed (within sheep), between goats and sheep and depending on the parasite species.

2 Objectives of the PhD project

The present thesis aims to unravel the genetic background of small rumenants resistance to GIN by exploring the mechanisms involved in resistance and susceptibility. It additionally aims to study phenotypic and genomic markers that could be used as biomarker in breeding for resistance. More specifically the objectives are:

1. Evaluate the pertinence of the immunoglobulin responses (especially IgA and IgE) against GIN and their potential use as biomarkers in breeding schemes. (Paper I).

2. Identify the molecular pathways involved in the response of Creole goats to GIN infection by analysing the transcriptome of abomasal mucosa and draining lymph nodes of infected versus non-infected and resistant versus susceptible kids (Paper II).

3. Identify the changes over time in the molecular pathways and immunity development in response of Creole goats to GIN infection by analysing the transcriptome of abomasal mucosa of resistant and susceptible kids at different time point post infection (Paper III).

4. Discover the genomic variants in the abomasal mucosa transcriptomes of Creole goats resistant or susceptible to *Haemonchus contortus* and characterized the variants identified (Paper IV).

3 Description of studies and main results

This thesis is comprised of four papers in which a systematic review and genomic information were employed to explore possible phenotypic and genomic markers for resistance to GIN and to understand the mechanisms involved in host resistance. In paper I, a total of 41 scientific publications on parasite resistance in sheep and goats were summarized to identify the main factors relevant for the design of such studies. Information extracted from the publications was further summarized to analyse relevant parameters of the immune system during parasite infection. In paper II, the gene expression profiles of resistant and susceptible goat kids were analysed after parasite infection. This study was further followed up in paper III in which the gene expression profile was studied at different stages after infection. The findings in this thesis should assists to develop appropriate small ruminant breeding strategies against GIN in the future.

3.1 Paper I

Immunoglobulins as biomarkers for gastrointestinal nematodes resistance in small ruminants.

A systematic review was conducted for the literature published until June 2019, which measured at least one immunoglobulin type during GIN infection in the small ruminants, goats and sheep. Figure 3 represents different steps for collecting literature and data from the scientific publications. The paper re-analysis and summarizes the literature findings on immunoglobulins response to GIN.

Results showed that immunoglobulins have good potential to be used as phenotypic markers for GIN resistance. For example, IgA level is a potential biomarker to breed for reduced parasite growth and fecundity. Meanwhile IgE

level and mast cells are potential biomarkers to breed for reduced parasite establishment and survival. Also other immune parameters were identified as potential biomarkers for the number of inhibited larvae. However, some factors were variable between the different literature sources which made results incomparable. We also highlight factors of the study design that should be taken into account to make future research more comparable. We identified for example the age of the animals, the infection experience and the type of infection (natural, single or trickle) as important factors of the environment, which have a large impact on the study outcome, but which vary largely between studies.

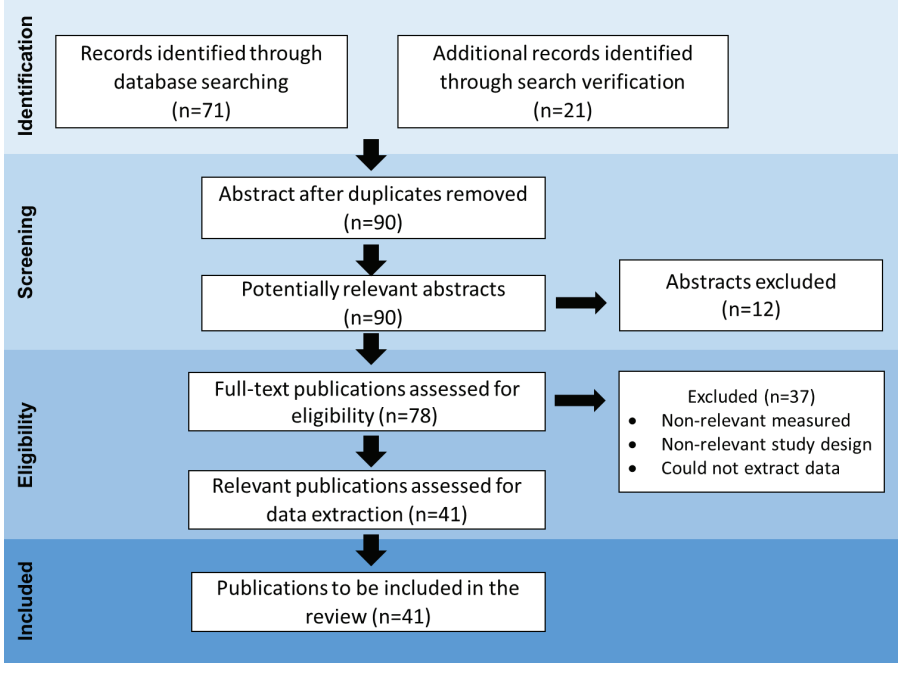

Figure 3. Steps for choosing included publications.

3.2 Paper II

Transcriptome variation in response to gastrointestinal nematode infection in goats.

An experiment to study molecular mechanisms of non-infected, resistant and susceptible Creole goats experimentally infected with *Haemonchus contortus* was conducted at the Research station PTEA (Plateforme Tropicale d'Expérimentation sur l'Animal, INRA, Guadeloupe). The study was based on

two diverse subpopulations from a Creole goat population, selected for resistance and susceptibility for *Haemonchus contortus* based on estimated breeding values for faecal egg count. Kids from the two parts of the population were infected with the parasite and samples were collected from them at different time points after two rounds of parasite infection. RNA-seq technology was used to conduct transcriptome profiling of the abomasal mucosa and lymph node tissues. Gene expression, gene ontology and pathway enrichment analysis were compared between non-infected, resistant and susceptible infected Creole goats at late infection (42 dpi).

Creole goats showed resistance to GIN infection through reducing worm fecundity and not worm burden. Comparing mucosal tissue from infected versus non-infected kids, 'Cell cycle' and 'cell death and survival' were the main identified networks. These indicate that at late infection stage the host response priority is to maintain the integrity of the mucosa. *TGFβ1* and MHC class I genes had a probable role in resistance to GIN infection.

3.3 Paper Ⅲ

Dynamic transcriptomic changes of goat abomasal mucosa in response to *Haemonchus contortus* infection.

The main results from paper II showed that maintain the integrity of the mucosa is the priority in host response at late infection stage. Therefore, another experiment was conducted to study the dynamics of the response of the abomasal mucosa from resistant and susceptible Creole goats experimentally infected with *H. contortus*. In this experiment whole transcriptome from abomasal mucosa were compared between resistant and susceptible infected kids at different time point of infection (0, 8, 15 and 35 dpi). The experimental design was as the one for paper II, except multiple time points of sample collection. Again RNA-seq technology was used to compare the transcription profiles of resistant and susceptible kids.

Innate (Th1) and adaptive (Th2) immune response was activated in response to infection. Results showed earlier immune response in resistant animals compared to susceptible ones. The mechanisms underline resistance were controlled through many genes which reflect the polygenic nature of the trait. *IL2RG, IL4R, STAT6, GATA3, CCR4, STAT3, RORC, TGFβ1* and *IL17F* genes showed an important role in determining animal response to GIN infection, which give them potential to be used in breeding scheme for resistance.

3.4 Paper Ⅳ

Genomic variants from RNA-seq for goats resistant or susceptible to gastrointestinal nematode infection.

The genomic variants between resistant and susceptible Creole goats in response to *H. contortus* infection were discovered from RNA-seq data of the previous experiment at four different time points post-infection (0, 8, 15 and 35 dpi). SNPs, insertions and deletions that distinguish the resistant and the susceptible kids were identified and characterized through functional analysis for the genes containing these variants.

The average number of SNPs that were identified per gene was double for resistant animals compared with susceptible one. Data from the resistant group contained more insertions and deletions among genes. MAPK signalling pathway, T cell receptor signalling pathway, hepatitis B and longevity regulating pathway were the top significant pathways that distinguish the resistant from the susceptible kids. 78% of genes in T cell receptor signalling pathway had genomic variants that distinguish the resistant from the susceptible animals. This study considered one of the first discoveries for genomic variants between resistant and susceptible animals at functional genes level which have potential to be used in breeding for GIN resistance.

4 General discussion

Evidence for the genetic variation in host resistance to GIN among small ruminant breeds (Zajac *et al.*, 1990; Baker *et al.*, 1998, 2003; Amarante *et al.*, 1999, 2005; Sayers *et al.*, 2007; Shakya, 2007; Bowdridge *et al.*, 2013) and within the animals of the same breed (Bambou *et al.*, 2013; McRae, Good, *et al.*, 2014; McBean *et al.*, 2016) rise the interest in control strategies based on the host immuno- genetics/genomics. Host resistance has been characterized by rapid genetic progress in small ruminant flocks both under research and commercial conditions (Morris *et al.*, 1997, 2000, 2005; Williams *et al.*, 2010; McRae, McEwan, *et al.*, 2014). Therefore, breeding for host resistance is considered a decisive method of GIN control. A good knowledge of the mechanisms underlying protective immunity in small ruminant is a prerequisite for the development of immune- genetics/genomics methods to control gastrointestinal helminths. A discussion of the host immune response to GIN, which are associated with resistance or susceptibility, and the genetic regulation mechanisms for immunity are summarized here. Besides, the impact of other factors such as chitinas and oxidative status is highlighted.

4.1 Host immunity against GIN

An infection with GIN larvae induces host response to control the infection. The development of immunity to GIN is complex and highly variable depending on host breed, the GIN specie and the intensity of infection (McRae *et al.*, 2015). Protective immunity to GIN is mediated, at least partly, by parasite-specific antibodies response (McRae *et al.*, 2015). Small ruminant antibody response includes IgG1, IgG2, IgM, IgA and IgE isotypes (Schallig, 2000). During the last decades many research groups have studied the possible role of these antibodies in immunity against GIN.

In article 1, we examined and summarized the role of parasite-specific antibody response. We summarized this role according to the three major mechanisms of immunity to GIN that have been described in sheep, prevention of establishment of most incoming infective larvae, suppressed GIN growth and therefore fecundity, and the expulsion of adult worms; or a combination of these mechanisms (McRae *et al.*, 2015). Reduced parasite establishment and survival is associated with IgE activity mainly against incoming third stage larvae (L3) in concert with mast cells as cross-linking of IgE on the mast cell surface leading to mast cell degranulation (Stear *et al.*, 2009; Murphy *et al.*, 2010) with more prominent response in previously infected animals (Huntley *et al.*, 1998). Reduced parasite growth and fecundity is associated with increased local IgA activity against fourth stage larvae (Stear *et al.*, 1995, 2004, 2009). Increased number of inhibited larvae is associated with IgG1 activity against the third stage larvae (Douch, Green and Risdon, 1994; Schallig, van Leeuwen and Hendrikx, 1995) beside IgA activity against the third and fourth stage larvae (Stear *et al.*, 2004, 2009). However, some studies in goats indicated that humoral response is not correlated with GIN resistance in goats (Bambou *et al.*, 2008; de la Chevrotière *et al.*, 2012; McBean *et al.*, 2016).

Eosinophils, mast cells and globule leukocytes (degranulated mast cells) have all been implicated as effector cells mediating resistance to GIN (Schallig, 2000; Arsenopoulos, Symeonidou and Papadopoulos, 2017). Eosinophils are a type of white blood cell and assumed to have a major role in the innate immune response. They have been reported to have a significant role in protection to GIN infections at least against *H. contortus* (Schallig, 2000; Arsenopoulos, Symeonidou and Papadopoulos, 2017). Eosinophilia have been correlated with protection against *H. contortus* in sheep (Balic, Cunningham and Meeusen, 2006; Robinson *et al.*, 2010; Shakya *et al.*, 2011). However, a relationship was neither found between the number of adult *T. circumcincta* and tissue eosinophilia (Henderson and Stear, 2006), nor between FEC of *T. circumcincta* and circulating eosinophil counts (Beraldi *et al.*, 2008). This is probably due to the fact that *T. circumcincta* causes little damage to the mucosal epithelium (Venturina, Gossner and Hopkins, 2013). In goats, blood eosinophil was also reported to increase significantly after infection with *H. contortus* (Bambou *et al.*, 2008) and to have negative correlation with FEC (de la Chevrotière *et al.*, 2012). The hyperplasia of mucosal mast cells is one of the most marked features of a GIN infection (Schallig, 2000; Arsenopoulos, Symeonidou and Papadopoulos, 2017). Mucosal mastocytosis, including globule leucocytes, was associated with GIN, which suggest that type I immediate hyper-sensitivity reactions are important in worm expulsion (Miller, 1984). In this context, significant increases of mast cell in the gastric lymph and globule leukocytes

were observed in infected and reinfected 'immune' sheep (Stear *et al.*, 1995; Huntley *et al.*, 1998). Similarly in goats, globule leukocyte had negative correlations with number of worm (Paolini *et al.*, 2003) and immature worm burden (Bambou *et al.*, 2013) after infection with *H. contortus*. Higher numbers of abomasal mucosal eosinophils, mast cells and neutrophils have been observed in infected compared to uninfected lambs, with higher level in resistant than susceptible breeds (Shakya *et al.*, 2011).

4.2 Regulation of host immune mechanisms

Although antibodies and mast cells have been reported to play the major role in the host control of parasite infection, these factors are regulated by the cytokine environment generated by activated T cells (Venturina, Gossner and Hopkins, 2013). Identifying the type and mechanism of T cell activation involved in the immunological regulation of infection is critical in understanding the host control of GIN infection.

4.2.1 Major Histocompatibility Complex (MHC I and II)

Presentation of antigens via Major Histocompatibility Complex (MHC) class I and class II molecules for recognition by specific T-cell receptors is central to T-cell activation (Vyas, Van Der Veen and Ploegh, 2008). MHC class I presents intracellular peptides at the cell surface of CD8+ T cells when intracellular pathogens such as viruses induce cellular expression of viral proteins. Some of these viral proteins are tagged for degradation, with the resulting peptide fragments entering the endoplasmic reticulum and binding to MHC class I molecules (Neefjes *et al.*, 2011). A MHC class II on the other hand presents peptides from extracellular pathogens at the cell surface of CD4+ T cells which help to trigger an appropriate immune response including localized inflammation or lead to a full-force antibody immune response due to activation of B cells (Vyas, Van Der Veen and Ploegh, 2008; Neefjes *et al.*, 2011).

One candidate region for genes involved in parasite resistance or susceptibility is the MHC. MHC class II regions have been associated with GIN resistance in different breeds of sheep (Schwaiger *et al.*, 1995; Outteridge *et al.*, 1996; Paterson, Wilson and Pemberton, 1998; Charon *et al.*, 2002; Sayers, Good, Hanrahan, Ryan, Angles, *et al.*, 2005; Stear, Innocent and Buitkamp, 2005). In this context, using transcriptional profiling of nematode-resistant and susceptible sheep lines, up-regulation of MHC class II genes was observed in resistant animals (Keane *et al.*, 2007). In a mouse model infected

with *Strongyloides venezuelensis*, MHC class II but not class I molecules were required to induce a predominantly immune response and to achieve efficient control of infection (Rodrigues *et al.*, 2009).

Our results from article 2 (Aboshady *et al.*, 2019) indicated that the top biological functions for the DEG identified from the comparison of lymph node tissue from resistant and susceptible goats were related to antigen processing and presentation of peptide antigen via MHC class I. The 'antigen processing and presentation of peptide antigen via MHC class I' was also reported as one of the major functional annotation cluster of genes differentially expressed in abomasal lymph nodes in sheep breeds known to differ in GIN resistance (Ahmed *et al.*, 2015). The implication of the MHC class I molecules in the mechanisms underlying genetic resistance to *H. contortus* was reported through an association between reduction in FEC and a homozygotes allele for the MHC class I (*OMHC1-188*) in sheep (Castillo *et al.*, 2011). A MHC class I antigen in close linkage disequilibrium with the DRB1 class II antigen, was associated with a 10-fold reduction in FEC following natural predominantly *Ostertagia circumcincta* infection in lambs (Stear *et al.*, 1996). The linkage disequilibrium between MHC class I and II antigen means that it is difficult to say which one is the causative for the FEC reduction.

Our results in goats and other results from previous studies in sheep (Castillo *et al.*, 2011; Ahmed *et al.*, 2015) suggest that MHC class I plays a role in resistance to GIN infection. This result is not expected from the previous known functions for MHC, that class I present in response to intracellular pathogens and class II present in response to extracellular pathogens (Neefjes *et al.*, 2011). Beside that MHC class II but not class I molecules are required for predominantly immune response and control of GIN infection in mice (Rodrigues *et al.*, 2009).

4.2.2 T cell receptors

The T cell receptor (TCR) is a complex of integral membrane proteins on the surface of T cells, which recognizes the antigens presented by MHC and plays a central role in the adaptive immune response (Vyas, Van Der Veen and Ploegh, 2008; Huse, 2009). Recently, TCR signalling has been linked to gene regulation through downstream pathways which modify gene expression (Huse, 2009).

Results from article 4 showed that the TCR signalling pathway was one of the top significant pathways identified for genes containing genomic variants from resistant animals. By examine genes involved in TCR signalling pathway we found that 78% of these genes have one or more genomic variants that exist

in resistant and not in susceptible animals. TCR signalling pathway was not identified previously as top significant pathway in studies comparing gene expression between resistant and susceptible animals. Despite that TCR signalling pathway was one of the immune pathways identified for immune genes containing SNPs in sheep chromosome 3 (OAR3) which were associated with GIN resistance (Periasamy *et al.*, 2014). In this study, they used a large number of animals (n = 713) which represent 22 breeds across Asia, Europe and South America. The results in this study align with our findings of the role of TCR signalling in the adaptive immune response against GIN infection and that genomic variants in genes involved in it affect animal immune response.

4.2.3 T helper (Th) cells and cytokines

On encountering a foreign antigen, MHC class I or II carrier molecules display the antigens to their cognate T cell receptor, which activates the naïve T cell and initiates the adaptive immune response. Consequentially, this results in release of cytokines, leading to both T cell differentiation and the proliferation of further T cells (McRae *et al.*, 2015).

The adaptive immune response against GIN has been studied extensively in rodent models (Miller, 1984; Sher *et al.*, 1990; Urban *et al.*, 1992, 1996; Finkelman *et al.*, 1997). As a consequence, our knowledge in host immune response comes mainly from these models. Traditionally, it has been accepted from studies on murine models that immunity is dependent on CD4+ T cell (Th0) activation which develops in two mainly distinct pathways, T helper type 1 (Th1) and type 2 (Th2) cell response based on the cytokines that they secrete (Mosmann *et al.*, 1986; Mosmann and Coffman, 1989). Th1 cells produce a number of cytokines principally interleukin 2 (IL-2), interferon-γ (IFN-γ) and tumor necrosis factor β (TNF-β) resulting in a cell mediated immune response (Schallig, 2000). Meanwhile, Th2 cells produce another number of cytokines such as IL-4, IL-5, IL-9, IL-13, IL-25 and IL-33 among others, which induce differentiation and maturation of intraepithelial mast cells, eosinophilia and goblet cell development (Mosmann and Coffman, 1989; Artis and Grencis, 2008; Li *et al.*, 2012). IL-4 and IL-5 induce an inflammatory response that is characterized by IgE production in case of IL-4 (Finkelman *et al.*, 1990) or eosinophilia in case of IL-5 (Coffman *et al.*, 1989; Sher *et al.*, 1990). Besides, IL-4 with IL-3 and IL-9 serve as a co-factor in the development of intestinal mucosal mast cells (Hültner *et al.*, 1990; Urban *et al.*, 1992). Meanwhile, IL-13 activates goblet cells leading to increases the secretion of mucus and prevents contact of parasites with the epithelial surface. Additionally, IL-13 and IL-4 activate macrophages that produce metabolic products to attack and stress

larval stage of GIN within the intestinal mucosa (Artis and Grencis, 2008). A typical Th2 response is characterized by increased immunoglobulin secretion by plasmocytes, in particular IgG1, IgA and IgE, and proliferation of eosinophils and mast cells.

Research using murine models has underlined the role of Th2 response and high levels of cytokines IL-4 and IL-13 with resistance of the host immune system against GIN infection, while a Th1 response with high levels of IFN-γ have been linked with susceptibility (Urban *et al.*, 1992; Maizels and Yazdanbakhsh, 2003; Anthony *et al.*, 2007). In general, Th1 response is activated during intracellular parasite infections, where IFN-γ is the predominant immune activator, while Th2 response is activated during extracellular parasite infections where IL-4 plays a prominent role in elevating humoral immune mechanisms (Urban et al., 1996).

Results from the systematic review in this thesis (article 1) showed that immunoglobulin response and therefore Th2 response differ between sheep breeds. There is strong suggestion that goats develop a different set of strategies to regulate GIN infections and to establish immunity, compared to sheep. Moreover, some goats appear to lack a functional IgA and eosinophil response against natural GIN infection. In this context, results from article 3 showed that the Th1 pathway was one of the top pathways identified in most of the comparison performed. Looking at differential of CD4+ T cell, signals for Th1 and Th2 activation were found in resistant animals when comparing them with susceptible animals. Results suggested that activation for Th2 genes is earlier in resistant goats compared to the susceptible ones. Altogether these results indicate that the Th2 response against GIN infection is less effective in goats than sheep and probably does not play the main role in the mechanism underlying genetic resistance in goats. While a Th1/Th2 balance could be more important than a Th2 response alone.

In ruminants, the view that a Th1 response is associated with susceptibility and a Th2 response with resistance, as well as their balance, consider an issue of conflict. For example, IFN-γ inhibited host protective responses to *Strongyloides papillosus* infection in cattle resulting in increased larvae survival (Nakamura *et al.*, 2002). A Th1 response was observed in susceptible sheep infected with *H. contortus* through an increased expression of TNF-α and IFN-γ (Zaros *et al.*, 2014). In addition, Th1 response was linked to susceptibility and Th2 response to resistance in reviewing genetic resistance of sheep to *T. circumcincta* (Venturina, Gossner and Hopkins, 2013).

On the contrary, another study supported a relationship between IFN-γ and resistance to GIN infection in Texel sheep (Sayers, Good, Hanrahan, Ryan and Sweeney, 2005). TNF-α and IFN-γ expression was increased after *H. contortus*

infection in sheep both in the abomasal mucosa and the draining lymph nodes (Pernthaner *et al.*, 2005, 2006; Robinson *et al.*, 2011). In the same context, infection with *Ostertagia ostertagi* in cattle resulted in decreased levels of IL-2 transcription and increased levels of IL-4 and IL-10 transcription. These observations are consistent with Th1 depression and Th2 activation; however these did not protect the calves against the *O. ostertagi* infection.

Another hypothesis support existing balance/ratio between Th1 and Th2 to express resistance genotype. A study showed an increased expression of IFN-γ and IL-12 despite a predominant Th2 response in immunized sheep during *H. contortus* infection (Meeusen, Balic and Bowles, 2005). Schalling (2000) suggested that the more important factor for the final outcome of the immune response is not the quantity of each cytokine but the ratio of the different cytokines. Our results from article 3 support this hypothesis.

4.2.4 Th17 responses and Regulatory T cells (Tregs)

Another distinct T cell category is Th17 cells which promote inflammation response through production of IL-17 and IL-21 cytokines (Venturina, Gossner and Hopkins, 2013). Inducing T cells to differentiate to Th17 instead of other T cell strains requires IL-23 stimulation following IL-6 and TGF-ß1 stimulation (Kimura and Kishimoto, 2010; Jin and Dong, 2013). IL-17 family members and IL-21 cytokines are known for their important in cleaning pathogens and inducing tissue inflammation at early infection (Korn *et al.*, 2009; McRae *et al.*, 2015). IL-17A and IL-17F mediate their immunological function by inducing pro-inflammatory cytokine, anti-pathogenic peptide and chemokine secretion by responder cells. The release of these pro-inflammatory molecules triggers the recruitment of innate immune cells to the site of infection and eliminate the pathogen (Jin and Dong, 2013).

Human patients with a genetic mutation in the STAT3 gene have defective IL-17A/F production and suffer from high susceptibility to infections from different pathogens (Milner *et al.*, 2008). Our results showed that the expression levels for genes controlling the Th17 response had a positive fold change for STAT3 and RORC genes in resistant compared with susceptible kids at 15 days post infection (dpi), and for IL17F at 35 dpi (article 3). In sheep research, Th17-associated genes have been associated with resistance to GIN at an early stage of infection (MacKinnon *et al.*, 2009). In contrary, increased expression of IL-6, IL-23A and IL-21 have been associated with susceptibility to GIN at 12 weeks after trickle infection (Gossner *et al.*, 2012). These results indicate the role of Th17 response in resistant to GIN at early stage of infection.

The host can control the immune response against parasite infection by the development of T regulatory cells (Tregs) (Venturina, Gossner and Hopkins, 2013). Tregs are known to have two main functions. Firstly, they have the ability to suppress the immune response with IL-10 and TGF-β cytokines after prolonged immune activation to manage inflammation and limit tissue damages (Tang and Bluestone, 2008). Secondly, Tregs are critical for the clinical outcome of GIN infection (Venturina, Gossner and Hopkins, 2013; Arsenopoulos, Symeonidou and Papadopoulos, 2017).

TGF-β is a multifunctional cytokine produced by all white blood cells lineages and best known for its regulatory activity and induction of peripheral tolerance (Nakao *et al.*, 1997). In our studies, we found that the 'TGF-β signalling pathway' in the top significant pathway for the different expressed genes in the comparison of mucosa samples between resistant and susceptible kids at 42 dpi (article 2). Moreover, *TGF-β1* was one of the first upstream regulator gene that was differently expressed in mucosa tissue of resistant versus susceptible and infected versus non-infected kids, with a prediction to be inhibited in resistant kids at 42 dpi (article 2). By studying the expression of *TGFβ1* at different time of infection (article 3), we found it to be significantly higher in resistant compared with susceptible kids at early time of infection (8 dpi) and then down regulated in resistant animals at late infection (35 dpi), which is in agreement with findings in article 2. In this context, TGF-β receptor 1 was highly expressed in lymph nodes of a susceptible sheep breed compared with a resistant sheep breed at 27 dpi with *H. contortus* (MacKinnon *et al.*, 2009). Recently it was found that modulate cytokines profile to increases the secretion of IL-10 and TGF-ß1 in goat monocytes contributes to induce an anti-inflammatory environment (Wang *et al.*, 2017). This confirms the role of Tregs in maintenance of immunological tolerance.

4.3 Other factors related to host control of infection

4.3.1 Chitinase and chitinase-like proteins

Chitinases are a group of digestive enzymes that break down glycosidic bonds in chitin, which is present in the exoskeletal elements of GIN and arthropods (Fuhrman and Piessens, 1985). A mice model showed that chitinases (C) and chitinase-like proteins (CLP) production is an important feature of Th2 immune responses during nematode infection (Nair *et al.*, 2005). In a recent review of the role of C/CLP in immune response, Lee et al. (Lee *et al.*, 2011) reported that C/CLP are produced by the host in the case of mammals as a

defense against infection. They can inhibit chitin-induced innate immune and injury responses. Simultaneously, enhance adaptive immune responses, thereby ensuring the development of selective antigen-specific immunity. C/CLP are further induced during the type 2 immune response, and have the ability to contribute in the production of TGF-β1 and also probably to healing and fibrosis (Lee *et al.*, 2011).

The chitinase-3 like 1 (Chi3L1) transcript was found to be upregulated early (day 5 post infection) in both the abomasum and gastric lymph nodes in response to a *T. circumcincta* challenge of previously infected sheep. But it was upregulated late (day 21 post infection) in the abomasum of naïve sheep (Knight *et al.*, 2007). Expression of the chitinase-3 like 2 (Chi3L2) has been observed in the abomasal lymph node of resistant and susceptible Blackface lambs infected with *T. circumcincta* in comparison to uninfected animals (Gossner *et al.*, 2013). Expression of the same gene (Chi3L2) has also been reported in the abomasum of 18 and 21 week old steers exposed to *O. ostertagi* (McRae, McEwan, *et al.*, 2014). These could indicate that C/CTP play a role in immune response in both susceptible and resistant animals.

Our results for transcriptomic changes of goat abomasal mucosa in response to *H. contortus* infection (article 3) did not show any signature for C/CTP mechanisms. However, looking at the gene expression level we found that expression of Chi3L2 was significantly higher in resistant and susceptible kids at 8 and 15 dpi in comparison to day 0. While the expression was still high in resistant kids at 35 dpi in comparison to day 0, it decreases in susceptible kids. This leads to a significant difference in the expression of Chi3L2 between the resistant and susceptible groups, being 32-fold higher in resistant group. This supports the previous finding, that C/CPT plays a role in immune response in both susceptible and resistant animals, with a new sign for difference in maintaining high level in resistant animals.

4.3.2 Oxidative status

Another significant factor that was reported in parasite control is the generation of host oxidants (Ingham *et al.*, 2008; Patel *et al.*, 2009; Arsenopoulos, Symeonidou and Papadopoulos, 2017). Oxidants that have been associated with GIN resistance include phagocytic oxidase (PHOX) (Dzik *et al.*, 2006), dual oxidase (DUOX) (Ingham *et al.*, 2008; Menzies *et al.*, 2010; Lees *et al.*, 2011) and inducible nitric oxide synthase (NOS2A) (Rajan *et al.*, 1996). Reactive oxygen and nitrogen species (RONS), generated by these factors, have possible roles in facilitating GIN expulsion through direct damages of parasitic tissues or lethality (Colasanti *et al.*, 2002; Lees *et al.*, 2011).

An increase in the reactive oxygen producer dual oxidase 1 (DUOX1) transcript was particularly marked high in resistant sheep following 3 days of *T. colubriformis* challenge in previously infected animals (Ingham *et al.*, 2008). The DUOX2 expression was found to be important in the sheep mucosal inflammatory responses to GIN infection as it raised from 3 d.p.i (Menzies *et al.*, 2010). In this context, an early response to *H. contortus* experimental infection in resistant sheep was marked by an increase in expression of host oxidant producing genes: the dual oxidase group (DUOX2/DUOXA2) during day 1 to day 7 compared to day 0 of infection (Lees *et al.*, 2011). During days 1 to 7 post-challenge, a cluster of four cytokines, IFN-ɣ, IL4, IL5 and TNF-α, showed strong positive correlation to a second cluster containing mast cells, eosinophils and globular leukocytes as well as the expression of DUOX2, DUOXA2 and GPX2 (Lees *et al.*, 2011). It is interesting that this study noted a positive association between IFN-ɣ (Th1 cytokine) and IL4 expression (Th2 cytokine). Again, this result raises the role of both Th1 and Th2 in host resistance to GIN infection.

On the other hand, host reactive oxygen and nitrogen intermediates display high reactivity and low specificity. Therefore, they can damage host tissues, leading to dysfunction of the immune response which explains the requirement for effective host antioxidant defenses for the development of immunity against GIN infection (Arsenopoulos, Symeonidou and Papadopoulos, 2017). It was demonstrated that the host antioxidant response to infection is specific to the time of challenge at the time when oxidants expulsion effect was finished (first 7 days of the infection) and resistance in sheep was established (Lees *et al.*, 2011). This involving an increase in the expression of the glutathione peroxidase family genes (glutathione peroxidase 3, glutathione reductase and glutamyl cysteine deoxygenase gene) at 28 dpi.

Looking at the genes expression from article 3, there were no differences in DUOX1, DUOX2, DUOXA2 expression between resistant and susceptible animals at 8 or 15 dpi. These genes showed down regulation in resistant compared to susceptible animals at 35 dpi. The same genes were differently expressed between abomasal mucosa of infected and non-infected animals at 42 dpi (article 2), showing down regulation in infected compared to non-infected animals. At the same time, both experiments (article 2 and 3) did not verify differences in antioxidant genes expression. Despite no differences in antioxidant gene expressions, oxidants play a role in response to GIN infection in both resistant and susceptible animals with difference in expression prolongation.

4.4 Breeding for resistance to GIN

Breeding for GIN resistance depending on genetic variation has been the subject of many review research articles (Schallig, 2000; Davies, 2006; Stear *et al.*, 2009; Bishop, 2015; McRae *et al.*, 2015; Zvinorova *et al.*, 2016; Arsenopoulos, Symeonidou and Papadopoulos, 2017). Selection for resistance has traditionally been based on quantitative measurements of phenotypic traits as we discussed in the general introduction. One of these quantitative phenotypic traits that has potential to be used in breeding for GIN resistance is the immunoglobulin level. In article 1 we discussed the role of each immunoglobulin for the resistance to GIN infection. CarLA saliva IgA antibody test is currently being marketed (CARLA® SALIVA TEST) as a powerful new tool for measuring parasite immunity in sheep (https://www.agresearch.co.nz/doing-business/products-and-services/carla-saliva-test/). Salivary IgA measurements have been used to calculate an Estimated Breeding Value (EBV) for Lleyn sheep in an ongoing project and results from selection using these EBVs is in the way (https://www.isage.eu/wp-content/uploads/No.6_ORC_NSA-_Assessing-parasite-resistance-on-three-sheep-breeds-in-the-UK_FINAL.pdf).

On the other hand, the identification of molecular markers is potentially a more reliable approach in breeding for GIN resistance (Venturina, Gossner and Hopkins, 2013; Zvinorova *et al.*, 2016). In article 2 and 3, we examine the transcriptome variation between resistant and susceptible Creole kids in response to *H. contortus* infection from abomasal mucosa and lymph node tissue at late infection (article 2) and from abomasal mucosa at 8, 15 and 35 dpi (article 3). The purpose was to compare the genes expressions between resistant and susceptible kids in response to infection and to identify the different mechanisms involved in the control of infection. This is considered a first step to identify possible genes to be used as potential molecular markers in breeding for resistance. Article 2 showed that MHC class I and *TGFβ1* genes have a major role in controlling GIN infection and infection consequences, which make them possible molecular markers. In this context, the implication of the MHC Class I molecules in the mechanisms underlying genetic resistance to *H. contortus* was reported through an association between reduction in FEC and a homozygotes allele for the MHC class I (*OMHC1-188*) in sheep (Castillo *et al.*, 2011). The same role for *TGFβ1* was previously reported in other study in goats (Bhuiyan *et al.*, 2017) and also a study on sheep infected with *H. contortus* (MacKinnon *et al.*, 2009). Article 3 confirms the previous finding for the relevance of the *TGFβ1* gene besides suggestions for other genes for possible use as molecular marker like *IL2R, TNF, IFN-γ, IL4R, STAT6, GATA3, STAT3*, or *RORC*. Interestingly QTL were reported near the *RORC* gene,

transcription factors controlling Th17 maturation and function, on sheep chromosome 1 (OAR1) (Ellis *et al.*, 2009; Gutiérrez-Gil *et al.*, 2009; Marshall *et al.*, 2009). *IL2RB, IFN-γ,* and *TNFA* were also reported as proximate genes to different QTL found on sheep chromosome 3 and 20 which have been associated with FEC (Benavides, Sonstegard and Van Tassell, 2016).

Results from differential gene expression studies during infection assist in understanding the differences in mechanism between resistant and susceptible animals and the genes involved in these differences. Nevertheless, for the purpose of practical selection, variants causing the difference in expression should be identified as this will offer better opportunities that information on gene expression, which might rely on infection experiments. Meaningful and easy accessible molecular markers will be more useful as practical tools for breeding purposes. In article 3 we found that *IL17F* had the most significant difference in expression between resistant and susceptible kids at day 0 of infection (uninfected), having an expression three times higher in resistant compared with susceptible kids. This gene would have therefore a potential to be used as biomarker in a selection program. *IL17A* and *IL17F* were reviewed as proximate genes located in genomic regions that were found on sheep chromosome 20 and were associated with parasite resistance in sheep (Benavides, Sonstegard and Van Tassell, 2016).

In article 4, we discovered genomic variants in the abomasal mucosa transcriptomes of Creole goats classified as resistant or susceptible to *H. contortus* and characterized the variants identified. This could help in the previous raised issue concerning the causative variants to be used in breeding programs. Results from this article showed that 78% of genes involved in T cell receptor signalling pathway have one or more genomic variants that exist in resistant but not in susceptible animals. These genomic variants could be the key for the difference in activated T cells between resistant and susceptible animals and therefore have a potential to be used in breeding for resistance against GIN. This is the first study to examine the genomic variants between resistant and susceptible animals to GIN using information at the transcriptome level. However, there is still need to confirm these variants and to examine if they exist at DNA level or if they are post-transcription variants.

5 Future perspectives

Our results indicated the important of early response to infection in resistance to infection. Hence, studying the differences between resistance and susceptible animals at very early infection could provide better understanding for the resistance mechanisms.

Currently, animal selection and the search for biomarkers depend on low FEC which increase host resistance to parasites. However, resilient animals are not targeted by this approach. Hence, there is a need to identify resilient animals, discovering genes or genetic markers associated with resilient and mechanisms involved to include it in selection programmes.

Furthermore, studies showed that nutrition could be used as control strategy for GIN infection. Studies to determine nutrigenomic effect on resistance to GIN infection should be performed. Studying metagenomics during infection could also provide better information on infection mechanisms and hence better development for control strategy. Selection for resistance and/or resilience to GIN is complicated and polymorphic trait. This highlights the need for non-genetics/genetics methods to complement each other to prevent and control infection.

6 Conclusions

One promising method to control GIN and reduce its negative impact is selecting animals with a high immunoglobulin response. We highlighted factors that differ across studies and affect the immune response to GIN infection. Of these factors, age of the animals, the infection experience and the type of infection should be taken into account when designing future studies. Beside the need to standardize/normalize the measurements of immunoglobulin concentrations to be comparable between studies.

Our results suggested that resistance in Creole goats mainly controlled through reduction in worm fecundity and not worm burden with a major regulator role for MHC class I and TGF-β genes. At late infection, the priority of the host response is to maintain the integrity of the mucosal barrier

Goats infected with *H. contortus* induced simultaneous upregulation of Th1 and Th2 immune response at the mucosal level of resistant animals. Our results indicated an earlier activation in Th2 immune response in resistant goats compared to the susceptible ones. Some genes like *IL2R, TNF, IFN-γ, IL4R, STAT6, GATA3, STAT3*, or *RORC* have potential to be used in breeding for GIN resistance.

Results verified the possibility to use RNA-seq data as an efficient method and great resource to detect genomic variants at functional genes level. Genomic variants in genes involved in T cell receptor signalling pathway plays a role in GIN resistance in goats.

This work provides valuable resources for genomic differences and molecular mechanisms of the host response to GIN infection in small ruminant. This serves as a basis towards developing genomic markers for GIN resistance.

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Popular science summary

Small ruminants are widespread all over the world. They are source of different goods and benefits ranging from food with precious animal proteins (meat and milk) to manure, fibre and skins, draught power in the highlands, food security and important non-market services like insurance, cultural and ceremonial purposes. The world's sheep and goat populations have increased steadily over the past decades, especially in developing countries. One of the main constraints on small ruminant production is the management of animal health. Infection with gastrointestinal nematode parasites has the greatest impact upon animal health and productivity. Anthelmintic treatments had been the main control strategy during the last decade leading to rise of anthelmintic resistance worldwide. Genetic selection for resistant animals is a promising sustainable strategy to control GIN infection.

This work aimed to understand the mechanisms involved in host resistance to GIN and explore possible phenotypic and genomic markers for resistance that could be used to develop appropriate small ruminant breeding strategies. We could show important factors for the design of future studies when summarizing the literature. Furthermore, we did identify relevant biologic pathways for the response to parasite infection in goats. Some of the information will add information to develop a potential selection of resistant goats in the future.

Firstly, we re-analyse and summarize the literature findings on immunoglobulin response to GIN. Immunoglobulins showed good potential to be used as phenotypic markers for GIN resistance. IgE level and mast cell for example could be used to breed for reducing parasite establishment and survival. IgA level has the potential to be used in breeding for reducing parasite growth and fecundity. And other immune parameters are potential biomarkers for the number of inhibited larvae. We highlight factors that should be taken into account to make future research comparable such as age of the animals, the infection experience and the type of infection (natural, single or trickle).

Secondly, we performed two experiments to study molecular mechanisms and genomic variants between resistant and susceptible Creole goats in response to *Haemonchus contortus* infection. In the first experiment, we compared transcriptome profiling of abomasal mucosa and lymph node tissues between non-infected, resistant and susceptible infected Creole goats. This breed showed resistance to GIN infection through reducing worm fecundity and not worm burden. Results indicated that at late infection stage the host response priority is to maintain the integrity of the mucosa. *TGFβ1* and MHC class I genes had a probable role in resistance to GIN infection. In the second experiment, we examined the host response at different time points of infection through studying the dynamic transcriptomic changes of the abomasal mucosa of resistant and susceptible infected Creole goats. Innate (Th1) and adaptive (Th2) immune response was activated in response to infection. Results indicated earlier immune response in resistant animals compared with susceptible ones. The mechanisms underline resistance were controlled through many genes. *IL2RG, IL4R, STAT6, GATA3, CCR4, STAT3, RORC, TGFβ1* and *IL17F* genes showed an important role in determining animal response to GIN infection, which give them potential to be used in breeding scheme for resistance.

Finally, we used RNA-sequencing data from the second experiment to discover the genomic variants in resistant and susceptible animals. We were able to identify single nucleotide polymorphisms, insertions and deletions in the resistant and in the susceptible groups and compare them. The distinguished variants between resistant and susceptible animals were characterized through functional analysis. One of the top significant pathways that were identified for genes containing genomic variants was T cell receptor signalling pathway. 78% of genes in this pathway had genomic variants in resistant and not in susceptible animals. This study considered one of the first discoveries for genomic variants between resistant and susceptible animals at functional genes level which have potential to be used in breeding for GIN resistance.

Synopsis de la thèse

Les petits ruminants sont présents partout dans le monde. Ils sont source de différents biens et avantages allant de la sécurité alimentaire, avec des protéines animales de qualité (viandes et lait), au fumier, aux fibres et aux peaux, et à d'importants services non marchands tels que les cérémonies. Les populations mondiales de moutons et de chèvres ont augmenté régulièrement au cours des dernières décennies, en particulier dans les pays en développement. L'une des principales contraintes à la production de petits ruminants est la gestion de la santé animale. Les infestations par des nématodes gastro-intestinaux (NGI) est la plus grande contrainte sur la santé et la productivité des animaux. Les traitements anthelminthiques avaient été la principale stratégie de lutte au cours des dernières décennies, entraînant une augmentation de la résistance aux anthelminthiques dans le monde entier. La sélection génétique d'animaux résistants est une stratégie durable prometteuse pour contrôler l'infection aux NGI.

Ce travail visait à comprendre les mécanismes impliqués dans la résistance de l'hôte aux NGI et à caractériser des marqueurs phénotypiques et génomiques de la résistance qui pourraient être utilisés pour développer des stratégies appropriées d'élevage de petits ruminants. Premièrement, nous avons ré-analysé et résumé les résultats de la littérature sur la réponse des immunoglobulines aux NGI. Les immunoglobulines ont montré un bon potentiel pour être utilisées comme marqueurs phénotypiques de la résistance aux NGI. Le niveau d'IgE sériques et les mastocytes pourraient être utilisés pour une sélection visant la réduction de l'installation et de la survie des parasites. Bien que le niveau d'IgA puisse être utilisé en sélection pour réduire la croissance et la fécondité des parasites. Les IgG1 avec IgA sont de bons biomarqueurs pour le nombre de larves inhibées. Nous mettons en évidence certains facteurs qui devraient être pris en compte dans les recherches futures, tels que l'âge des animaux, l'expérience de l'infestation et le type d'infestation (naturelle, expérimentale unique ou répétée).

Deuxièmement, nous avons réalisé deux expérimentations pour étudier les mécanismes moléculaires et les variants génomiques entre les chèvres Créole résistantes et sensibles en réponse à une infection à *Haemonchus contortus*. Dans la première expérience, nous avons comparé les transcriptomes de la muqueuse abomasale et des ganglions lymphatiques entre des chèvres Créole infestés et non infestés, résistantes et sensibles. Cette race a montré une résistance à l'infestation aux NGI en réduisant la fécondité des vers et non la charge parasitaire (nombre de vers). Les résultats ont indiqué qu'à un stade tardif de l'infestation, la priorité de la réponse de l'hôte est de maintenir l'intégrité de la muqueuse. Les gènes TGFβ1 et MHC classe I ont probablement joué un rôle dans la résistance à l'infestation par *H. contortus*.

Dans la deuxième expérimentation, nous avons examiné la réponse de l'hôte à différents moments de l'infection en étudiant la dynamique des changements du transcriptome de la muqueuse abomasale de chèvres Créole infestées résistantes et sensibles. La réponse immunitaire innée (Th1) et adaptative (Th2) ont été activées de manière concomitante en réponse à l'infestation. Les résultats ont indiqué une réponse immunitaire plus précoce chez les animaux résistants. Les mécanismes sous-jacents la résistance étaient contrôlés par de nombreux gènes. Les gènes IL2RG, IL4R, STAT6, GATA3, CCR4, STAT3, RORC, TGFβ1 et IL17F ont montré un rôle important dans la réponse animale à l'infestation, ce qui leur donne le potentiel d'être utilisés dans le schéma de sélection pour la résistance.

Enfin, nous avons utilisé le séquençage d'ARN de la deuxième expérimentation pour identifier les variants génomiques chez les animaux résistants et sensibles. Nous avons pu identifier les SNP, les insertions et les délétions chez les résistants et les sensibles. Les variants distinguant les animaux résistants et les sensibles ont été caractérisés par une analyse fonctionnelle. L'une des principales voies importantes identifiées pour les gènes contenant des variants génomiques était la voie de signalisation des récepteurs des lymphocytes T. Près de 78% des gènes de cette voie présentaient des variants génomiques chez les animaux résistants comparés aux sensibles. Cette étude est l'une des premières découvertes de variants génomiques entre les animaux résistants et sensibles identifiés dans le transcriptome qui pourraient être utilisées dans la sélection pour la résistance aux NGI.

Populärvetenskaplig sammanfattning

Små idisslare finns spridda över hela världen. De är källan till olika varor och tjänster. Exempel på varor och tjänster är kött, mjölk, gödsel, fiber, skinn, dragdjur i högländerna och livsmedelssäkerhet. Dessutom kan det finnas viktiga icke-marknadstjänster som försäkring, kulturella och ceremoniella ändamål. Antalet får och getter har ökat stadigt under de senaste årtiondena, särskilt i utvecklingsländerna. En av de viktigaste begränsningarna för produktion med små idisslare är hanteringen av djurhälsa. Infektion med GIN har störst inverkan på djurhälsa och produktivitet. Gastrointestinala nematoder (GIN) är maskar som är parasiter i magen och tarmen. Behandlingar med avmaskningsmedel har varit den viktigaste kontrollstrategin under det senaste decenniet. Detta har lett till ökad resistens mot avmaskningsmedel över hela världen. Genetiskt urval för resistenta djur är en lovande hållbar strategi för att kontrollera GIN-infektion.

Detta arbete syftade till att förstå de mekanismer som är involverade i värdens motstånd mot GIN. Vi vill dessutom utforska möjliga fenotypiska och genomiska markörer för resistens som kan användas för att utveckla lämpliga avelsstrategier för små idisslare. När vi sammanställer litteraturen kan vi visa viktiga faktorer för utformningen av framtida studier. Dessutom identifierade vi relevanta biologiska mekanismer för svaret på parasitinfektion hos getter. En del av informationen kommer att kunna användas för att utveckla avel av resistenta getter i framtiden.

Först analyserar och sammanfattar vi resultat från litteraturen om hur immunglobuliner ändras som svar på GIN. Immunoglobuliner visade god potential att användas som fenotypiska markörer för resistens mot GIN. IgE-nivå och mastcell kan till exempel användas för att avla för minskad bildning och överlevnad av parasiter. IgA-nivå har potential att användas i avel för att minska parasiters tillväxt och fruktsamhet. Andra immunparametrar är potentiella markörer för antalet inhiberade larver. Vi visar faktorer som bör beaktas för att göra framtida forskning jämförbar. Dessa faktorer är djurens

ålder, erfarenhet av infektion och typen av infektion (naturlig, enstaka eller upprepad).

Sedan utförde vi två experiment för att studera molekylära mekanismer och variation i DNA mellan resistenta och mottagliga kreolska getter som svar på infektion med *Haemonchus contortus*. I det första experimentet undersökte vi RNA i buksslemhinnor och lymfkörtvävnader. Detta jämförde vi mellan icke-infekterade, resistenta och mottagliga infekterade kreolska getter. Denna ras visade resistens mot GIN-infektion genom att minska maskens fruktsamhet men inte maskbelastning. Resultaten indikerade att värdens prioritet för respons vid sent infektionsstadium är att behålla slemhinnans integritet. Gener i MHC-klass I hade en sannolik roll i resistensen mot GIN-infektion. I det andra experimentet undersökte vi värdens respons vid olika tidpunkter för infektion genom. Vi gjorde det genom att studera de dynamiska förändringarna av RNA i buksslemhinnan hos resistenta och mottagliga infekterade kreolska getter. Medfött (Th1) och adaptivt (Th2) immunsvar aktiverades som svar på infektion. Resultat indikerade tidigare immunsvar hos resistenta djur jämfört med mottagliga. Mekanismerna som understryker resistens kontrollerades genom många gener. Generna IL2RG, IL4R, STAT6, GATA3, CCR4, STAT3, RORC, TGFB1, och IL17 hade en viktig roll i att bestämma djurens svar på GIN-infektion. Detta ger dem potential att användas i avel för resistens.

Slutligen använde vi data från sekvensering av RNA från det andra experimentet för att upptäcka variation i DNA hos resistenta och mottagliga djur. Vi kunde identifiera utbytta DNA-bokstäver, samt extra eller saknade DNA-bokstäver. Vi gjorde detta i de resistenta och i de mottagliga grupperna och jämförde dem. De olika varianterna i resistenta och mottagliga djur beskrevs med analys av geners funktion. En av de viktigaste reaktionsvägarna som identifierades för gener som innehöll variation var för signalering med T-cellreceptorer. 78% av generna i denna väg hade variation i DNA hos resistenta men inte hos mottagliga djur. Denna studie är en av de första som hittar skillnader i DNA mellan resistenta och mottagliga djur på funktionell gennivå som har potential att användas i avel för GIN-resistens.

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Last but not least, as The Quran mentioned “Say: O my Lord! Increase me in knowledge.” (TaHa: 114) ﴿وَقُلْ رَبِّ زِدْنِي عِلْمًا﴾

Individual training plan (ITP)

Training (30 ECTS minimum)		
Mandatory courses	**Where/when**	**ECTS**
EGS-ABG research school "emerging technologies"	Wageningen university, The Netherlands February 13-17th, 2017	1.5
Philosophy of Science	SLU, Sweden March 6th – April 6th, 2017	3.0
How to write and publish a scientific paper	SLU, Sweden April 20th –May 23rd, 2017	3.0
Research ethics for PhD students	SLU, Sweden Sep. 12- 27th 2017	2.0
EGS-ABG research school "Animal 4D"	AgroParisTech, France May 28th – 1st June 2018	2.0
Advanced scientific courses (≥ 18 ECTS)		
From Genome Sequence to Genomic Selection	SLU, Sweden April 4-8th, 2016	5.0
Introduction to programming In R	SLU, Sweden May 9 -13rd, 2016	2.0
Genetic Epidemiology of infectious diseases in Livestock	SLU, Sweden May 15-19 th, 2017	3.0
Introduction to Bioinformatics using NGS data	SciLifeLab, Uppsala Nov. 27 th – Dec. 1 st 2017	2.0
Statistics III: Regression analysis	SLU, Sweden Jan 16 - Feb 16, 2018	4.0

Analysis of livestock metagenomics datasets	Centre INRA Antilles-Guyane, Guadeloupe, France. May 13-17th, 2019	1.5
How to write your first grant application	SLU, Sweden January 15-17th, 2020	1.0
Total credits (≥ 30 ECTS)		**30**
Dissemination of knowledge		
International conferences	**Where/when**	
Transcriptome variation in response to gastrointestinal nematode infection in goats. Oral presentation at 69th Annual Meeting of the European Federation of Animal Science (EAAP).	Dubrovnik, Croatia. August 2018.	
Dynamic transcriptomic changes of goat abomasal mucosa during an experimental *Haemonchus contortus* infection in resistant and susceptible genotypes. Poster presentation at 37th International Society for Animal Genetics (ISAG) Conference.	Lleida, Spain. July 2019.	
Oral presentation at 21st seminar of Animal Genetics Division, INRA.	Paris, France. May 2018.	

Appendix 1. Summary of molecular genetic markers associated with GIN resistance in small ruminants.

Species	Markers	Parasite(s)	Breed	Chromosome (associated trait)	References
Goats	sM (3, MHC)	*T. colubriformis*	Australian Angora and Cashmere	23 (FEC, EOS)	(Bolormaa *et al.*, 2010)
	M (101, 29 chr.)	Mixed	Creole	22, 26 (FEC), 7, 8, 14 (EOS), 5, 9, 21 (PCV) 6 (BW), 1, 3, 10, 26 (IgE)	(De La Chevrotière *et al.*, 2012)
	SNP (50k)	*H. contortus*	Creole	4, 6, 11, 17 (FEC)	(Silva *et al.*, 2018)
Sheep	sM (OLA antigens)	Mixed	Romney	20 (FEC, Peps)	(Douch and Outteridge, 1989)
	sM (OLA, 9 haplorypes)	*H. contortus*	Romanov	20 (FEC)	(Luffau *et al.*, 1990)
	sM (DRB1 alleles)	Mixed predominant *T. circumcincta*	Scottish Blackface	20 (FEC)	(Schwaiger *et al.*, 1995)
	sM (10 lymphocyte antigens)	Mixed predominant *T. circumcincta*	Scottish Blackface	20 (FEC)	(Stear *et al.*, 1996)
	sM (2, MHC)	Mixed predominant *T. circumcincta*	Scottish Blackface	20 (FEC)	(Buitkamp *et al.*, 1996)
	sM (5, MHC)	Strongyle	Soay	20 (FEC)	(Paterson, Wilson and Pemberton, 1998)
	sM (14, IFNɣ)	Mixed	Romney	3 (FEC)	(Paterson *et al.*, 2001)
	sM (3, IFNɣ)	*T. circumcincta*	Soay	3 (FEC)	(Coltman *et al.*, 2001)

Species	Markers	Parasite(s)	Breed	Chromosome (associated trait)	References
Sheep	sM (IgE gene)	*T. colubriformis*	Merino	18 (FEC, confirmation filled for another flock for *T. colubriformis* or *H. contortus*)	(Clarke *et al.*, 2001)
	sM (18 on OAR1)	*T. colubriformis* & strongyle	Romney × Coopworth	1 (FEC)	(Diez-Tascón *et al.*, 2002)
	sM (7, OAR5)	Mixed predominant *H. contortus*	Corriedale and Polwarth	5 (FEC)	(Benavides *et al.*, 2002)
	sM (6, OAR20)	*H. contortus*	Rhönschaf	20 (Haematocrit, IgL, FEC)	(Janßen *et al.*, 2002)
	sM (DRB1 alleles)	Mixed	Polish Heath	20 (FEC)	(Charon *et al.*, 2002)
	M (133, 26 autosornas)	*T. colubriformis*	Peppin Merino	1*, 3*, 6, 11*, 12*, 24* (FEC)	(Beh *et al.*, 2002)
	sM (6, OAR20)	*H. contortus*	Merinoland	20 (FEC, IgL)	(Janßen *et al.*, 2004)
	M (165, 26 autosornas)	*T. columbriformis*	Churra	1, 6, 14, 20 (FEC) 1, 9 (IgA)	(Arranz *et al.*, 2004)
	sM (IFNɣ, 4 haplotypes)	*Trichostrongyle* & *Nematodirus*	Texel and Suffolk	3 (FEC in Texel)	(Sayers, Good, Hanrahan, Ryan and Sweeney, 2005)
	sM (DRB1 alleles)	*Trichostrongyle spp.*	Suffolk and Texel	20 (FEC in Suffolk)	(Sayers, Good, Hanrahan, Ryan, Angles, *et al.*, 2005)
	M (203 on 9 chr)	*Trichostrongyle spp.*	Romney × Coopworth	2, 8, 11 (N. adult), 23 (IgG, IgE)	(Crawford *et al.*, 2006)
	M (139 on 8 chr)	Mixed (*Strongyles, Nematodirus*)	Scottish Blackface	2, 3, 14, 20 (FEC) 3, 20 (IgA)	(Davies *et al.*, 2006)
	M (251,whole genome)	*Strongyle*	Soay	1, 6, 12 (FEC)	(Beraldi *et al.*, 2007)

Species	Markers	Parasite(s)	Breed	Chromosome (associated trait)	References
Sheep	sM (3 on OAR5 & 4 on OAR20)	Mixed predominant *H. contortus*	Corriedale and Polwarth	5 (FEC)	(Benavides *et al.*, 2009)
	M (27 on OAR1 & 28 on OAR3)	*H. contortus*	Indonesian Thin Tail × Merino	1, 3 (FEC)	(Ellis *et al.*, 2009)
	M (181,whole genome)	*T. circumcincta*	Spanish Churra	1 (IgA), 1, 6, 10, 14 (FEC)	(Gutiérrez-Gil *et al.*, 2009)
	M (140 to 177, whole genome)	*H. contortus*	Merino	1, 2, 3, 4, 6, 7, 8, 9, 10, 11, 12, 15, 16, 18, 20, 21, 22, 24, 25, 26, X (FEC)	(Marshall *et al.*, 2009)
	M (55 on 8chr)	*T. colubriformis*	Romney-Merino Backcross	3 (FEC, EOS), 21 (EOS), 22 (FEC)	(Dominik *et al.*, 2010)
	sM (12 on OAR3 and OAR14)	*Nematodirus* and *Strongyles*	Suffolk and Texel	3 (FEC), 14 (FEC)	(Matika *et al.*, 2011)
	M (172, 25chr.)	*H. contortus*	Red Maasai × Dorper Backcross	3, 6, 14 (FEC), 22 (FEC, PCV)	(Silva *et al.*, 2011)
	SNP (50k)	*T. colubriformis* then *H. contortus*	Mixed breed	1, 2, 4, 5, 9, 13, 15, 20, 26 (FEC *T. colubriformis*) 1, 17, 18 (FEC *H. contortus*)	(Kemper *et al.*, 2011)
	M (172, 25 autosomes)	*H. contortus*	Red Masaai, × Dorper	2 (FEC, PCV, WC), 4 (FEC), 10 (FEC), 12 (WC), 18 (WC), 22 (WC), 23 (FEC, WC), 25 (WC), 26 (ADG, LWT, PCV, FEC, WC, AFWL, EPW)	(Marshall *et al.*, 2012)

Species	Markers	Parasite(s)	Breed	Chromosome (associated trait)	References
Sheep	M (160) & SNP (50k)	*H. contortus*	Romane × Martinik Blackbelly Backcross	1 (PCV), 2 (PCV, SexR), 3 (FEC, WB) 4 (Len, pH), 5 (FEC, IgG, WB, PCV) 7 (FEC, Len), 9 (FEC), 10 (Peps) 12 (FEC, PCV, pH), 13 (FEC, IgG) 14 (Peps), 15 (FEC), 16 (FEC, WB) 17 (pH, PCV), 18 (Len), 19 (Len), 20 (FEC), 21 (FEC, Peps), 23 (FEC, WB), 25 (SexR, PCV)	(Sallé *et al.*, 2012)
	SNP (n=192)	*Strongyle*	Soay	No clear association with FEC/ specific antibodies and antinuclear antibodies	(Brown *et al.*, 2013)
	SNP (50k)	Mixed predominant *T. circumcincta*)	Scottish Blackface	6 (FEC, BW*), 14 (FEC), 21 (BW) 1* (FEC), 2* (FEC), 4* (FEC, IgA), 20* (FEC)	(Riggio *et al.*, 2013)
	SNP (41 on 38 genes)	Mixed	22 breeds	3 (FEC, PCV, LWT), 13 (PCV)	(Periasamy *et al.*, 2014)
	SNP (50k)	Mixed (*Nematodirus and Strongyles*)	Scottish Blackface, Sarda-Lacaune, Martinik Blackbelly-Romane Backcross	4, 12, 14, 19, 20 (FEC)	(Riggio *et al.*, 2014)
	SNP (50k)	Mixed predominant *H. contortus*	Red Maasai-Dorper Backcross	2*, 6*, 11*, 12*, 15* (FEC) 5*, 7*, 15*, 17*, 26* (PCV) 7*, 8*, 14*, 15*, 17* (LWT)	(Benavides *et al.*, 2015)
	SNP (50k)	*T. circumcincta*	Spanish Churra	6, 8, 22 (LA) 8, 10, 11, 12, 14, 15, 25 (GWAS)	(Atlija *et al.*, 2016)

sM, specific marker; M, microsatellite; SNP, single nucleotide polymorphism; chr., chromosome; FEC, faecal egg count; PCV, packed-cell volume; WB, worm burden; SexR, sex ratio in adult worm population; Len, female worm length; pH, abomasal pH; Peps, pepsinogen; WC, worm count; AFWL, adult female worm length; EPW, eggs per worm; LWT, live weight; ADG, average daily gain; LA, linkage analysis; GWAS, genome wide association selection. * Suggestive marker.

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The aim of this thesis was to unravel the genetic background of small ruminants resistance to gastrointestinal nematodes by exploring the mechanisms involved in resistance and susceptibility. It additionally aims to study phenotypic and genomic markers that could be used as biomarker in breeding for resistance to gastrointestinal nematodes.

Hadeer Aboshady received her doctoral education from: the Department of Animal Breeding and Genetics (SLU); AgroParisTech (France); and INRA (France). She obtained two MSc, one from the Polytechnic University of Valencia (Spain) and another MSc and BSc from the Cairo University (Egypt).

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