final report

Project code: B.AHE.0325

Prepared by: Professor David Emery
on behalf of the project co-authors: Drs Collette Britton, Brown Besier, Alasdair Nisbet, Thomas McNeilly, David Smith, Michael Stear and Ms Jill Lyon
The University of Sydney, Sydney School of Veterinary Science

Date published: 26th June 2019

PUBLISHED BY
Meat and Livestock Australia Limited
Locked Bag 1961
NORTH SYDNEY NSW 2059

The potential for vaccines against gastrointestinal nematodes of small ruminants

Meat & Livestock Australia acknowledges the matching funds provided by the Australian Government to support the research and development detailed in this publication.

Abstract

Gastrointestinal nematode (GIN) parasites cost the Australian sheep and goat industries around AUD 450M pa (Lane et al., 2015; MLA report B.AHE.0010), encompassing lost production and control measures and amounting to around 8% of the value of the Industry. With the dual aim to reduce production losses caused by scour worms in the livestock industries and to develop and maintain capacity and capability in parasitological research for livestock, this project reviewed the past and current research landscape regarding production of vaccines against scour worms, particularly *Trichostrongylus* spp and *Teladorsagia circimcincta.* The inability of past research to deliver scour-worm vaccines with reliable and reproducible efficacy has been due in part to gaps in knowledge concerning: (1) the intricacies of the host-parasite interaction leading to the development of (Th2, allergy-type) immunity, (2) the definition of an optimal suite of parasite antigens, and (3) the rational formulation and administration of ruminant vaccines to induce protective immunity against GIN at the site of infestation.

Recent 'omics developments and approaches enable more systematic analyses for each of 1-3 above. The GIN genomes are reaching completion, facilitating the "reverse vaccinology" approaches used successfully for the *R.australis* vaccine (Tabor 2019) for cattle tick, while methods for gene silencing and editing in GIN have been developed for screening and validation of potential vaccine antigens. As detailed in the project report, several areas require further development as part of a co-ordinated, integrated research approach (Section 7).

The review envisages that efficacious scour-worm vaccine(s) would be adopted similarly to "Barbervax ®" within integrated parasite management (IPM) schemes. In this process, vaccines would effectively parallel the use of resistant animals and also serve to reduce the frequency of drenching and the availability of parasites on pasture. Modelling of these aspects of integration, efficacy and operation need to be updated since the 1990s when the efficacy data shown in Figure 7 were derived.

The final section of the review (Section 7) proposes a staged approach to the implementation of an integrated reseach program. Given the 3-pronged interactive model, this is likely based on a "consortial" or multi-institutional (or multinational), collaborative operation encompassing a decade of sustainable funding. This is particularly needed to address the decline of career-based, parasitological expertise over the past 2 decades, and to ensure optimal chances of success.

Executive summary

Background (Section 1):

Gastrointestinal nematode (GIN) parasites cost the Australian sheep and goat industries around AUD 450M pa (Lane et al., 2015), encompassing lost production and control measures and amounting to around 8% of the value of the Industry. Apart from *Haemonchus contortus* (*Hc*; Barber's pole worm) a significant proportion of this cost is attributable to the combined impact of 2 other genera of "scour worms", including the abomasal parasitic species *Teladorsagia (Tel) circumcincta* ("small brown stomach worm") and *Trichostrongylus (T) axei* ("stomach hair worm") and small intestinal GIN, *T.colubriformis* and *T. vitrinus* ("black scour worms"). Several other scour worms of lesser importance, including *Nematodirus* spp ("Thin-necked intestinal worm"), Cooperia spp. ("intestinal hair worms"), and the large intestinal nematodes *Chabertia (C) ovina* ("large-mouth bowel worm") and *Oesophagostomum* spp ("large bowel worms") add to the production losses from the 3 major genera. Mixed infections are the norm, the proportional compositions varying between regions and enterprises, largely defined by the abundance and annual distribution of rainfall.

Control of GIN (Section 1-2):

Current best practice control measures to prevent the economic losses associated with scour worm infections revolve around the "prevention-detection-response" approach in which strategic use of anthelmintic treatments is key and is incorporated with the use of pasture management, breeding programs for natural resistance or resilience, nutritional management and effective diagnosis and drench efficacy testing. These are each integral components in successful, regional, integrated control programs (IPMs). Recently, with the development of anthelmintic resistance against all currently-available products, the successful introduction of a vaccine (Barbervax) has enhanced control of the barber's pole worm *Haemonchus contortus (Hc)*. Although highly effective, this vaccine does not control other species of worms, including the scour worms, which have a different host-parasite relationship (though there is anecdotal evidence that black scour worm appears easier to control after adoption of "Barbervax ™" to control *Hc*). In addition, the approach taken to develop Barbervax has not been successful for scour worms so vaccines for these species will require several components to be developed and rationally integrated into a vaccine development program. It is likely that specific vaccines will need to be developed to control each species, though it is recognised that a "pan-species" vaccine would be the ideal product.

Over the past decades, parasitological expertise has been diminishing in Australia through a combination of successful IPM programs, new and effective drenches, changes to research and industry priorities (esp genetics and marketing), and market returns on-farm driving enterprise changes to more mixed farming and dual-purpose sheep. In fact, many IPM programs are still based on seasonal data for pasture L3 populations from many decades ago and adoption is suboptimal (http://www.wormboss.com.au/news/articles/general/national-survey-highlights-parasite-control-practices.php). However, recent wool price increases have prompted a resurgence of interest in sheep, and restraints to their health and welfare, including parasites. With ongoing and growing concerns over the re-emergence of widespread anthelmintic resistance in *Telc* and *Tc,* and more erratic seasonal weather, refugia-based strategies such as targeted drenching have been introduced to IPM programs to prolong the efficacy of drenches.

Development of parasite immunity (Section 2):

Another strategy to reduce the frequency of drenching is to stimulate acquired immunity or resistance. Immunity to GIN develops slowly with age, taking around 4-6 months to become fully effective with continuous ingestion of parasites and adequate nutrition. Consequently, immunity wanes when parasites on pasture decline seasonally or during periods of stress, including parturition and lactation, and drought. Immunity appears to develop initially against ingested larvae, preventing establishment, then with increasing intensity, retard parasite development, decrease egg production and fertility and finally, expel adult/established worms. It is well appreciated that "resistant" sheep genotypes develop effective immunity faster, limiting the numbers of establishing worms and suffering lower productivity losses. However, collateral damage from the immune response has been widely reported, so to avoid untoward damage from "immune scours" and "immune-mediated pathology" causing production losses, vaccines would be ideally directed to early stages of the parasite (i.e. third-stage larvae, L3/early L4). As demonstrated for *Hc*, vaccines also integrate readily into IPM programs to provide respite for chemical control, effectively complementing the strategy for "breeding resistant sheep". At this point, immunisation experiments with irradiated (attenuated) larvae have shown that immunity can be generated against all 6 major GIN, but this level of protection has not been generated against scour worms with subunit vaccines to date.

Towards GIN vaccine development (Sections 3-5):

There are 3 major components to the development, formulation and delivery of a successful vaccine. For GIN, these are:

1. knowledge of the exact nature of the protective responses to be induced or replicated by the vaccine, so as to be effective against the parasite by mimicking naturally-acquired immunity;
2. selection of the most appropriate suite of "protective antigens" inducing effective immunity; and,
3. knowledge of the ability of different vaccine formulations (+/- adjuvants) to induce potential protective immune responses against GIN in sheep mucosae, so that the most "rational" combination of antigen, delivery agent and mode of delivery can be used to optimise success.

The approaches to these 3 components are outlined in the Schematics (Figures 4-6). Several technical advances are in place or require development to facilitate the approach. These are:

- fully annotated genomes for *Tel c* and *Tc* to enable identification of antigens and pathways in the life cycle of the parasites;
- gene knockout technology to identify genes essential for parasite development/survival;
- improved tissue culture systems for worm and host tissues, to enable high throughput validation of worm antigens and effects on parasite metabolism, development and survival *ex vivo* and *in vitro*, and analyses of host-worm interactions (and the genes/ pathways involved).

To address (1): in general terms, we know that "Th2 allergic-type" responses need to be induced for a conventional protective immune response that operates against scour worms in sheep (**Section 2**). However, the main targets of this immune response in the worms are poorly understood at this stage and methods for inducing the appropriate parts of the immune system through vaccination are also lacking. The review proposes that a systematic, dynamic investigation of the host-parasite interaction *in vivo* during the 3-6 weeks taken to develop immunity and worm patency (Figure 5). A current approach has been initiated by Moredun researchers for *Tel c,* to identify the important aspects of the host-parasite interface. Building on these experiences, a similar approach would be envisaged for *Tc*.

To address (2): many vaccine "candidate" antigens have been identified, purified and trialed as native or recombinant vaccines against *Telc* and *Tc*; to date without sufficiently reliable or a reproducible efficacy of >70% against worm counts or faecal worm egg counts (FWEC) (**Section 4**). While this has often been a combination of all 1-3 limitations above, there is no shortage of potential antigens derived from analyses of immune vs susceptible reagents. However, a new approach is afforded for scour worms. It is likely that new vaccines for scour worms will require several components to be developed through a reverse vaccinology approach and rationally integrated into the vaccine development program (1-3 above; Figures 4, 6; **Section 5**). Once these antigen/ pathway targets in the worms have been identified (1), a screening method is needed to allow the identification of the best targets from potentially large numbers of candidates at the start of the process. Currently that would rely on screening in the animals but the development of novel methods to do this in the laboratory would be faster, reduce potential ethical limitations and be more cost-effective, and a set of potential techniques to address this is presented in the review (**Section 5**). A prerequisite is an appreciation of what has already been attempted for these and other species and what current and emerging technologies might be employed to produce and evaluate novel vaccines. These include new 'omics technologies as well as antigen validation by methods such as gene knock-out, silencing or field trials as detailed in the review. Each of these aspects is addressed in the review and, although there is substantial knowledge in most of these areas, fundamental gaps exist in knowledge, available resources and technologies (above) to allow a fully functional reverse vaccinology approach.

To address (3): we know how to induce high levels of "protective" antibody in sheep using a range of "immunostimulants" or adjuvants (**Section 3**). What is not known is exactly HOW the host-parasite interaction over several weeks manages to generate the protective immunity to prevent parasitism (Section 4.2.2). Without that precise knowledge, we concomitantly do not know how to induce such (mucosal) immunity using a relevant vaccine formulation. While we recommend the kinetic analysis of the host-parasite interaction to provide insights into (1), a similar approach is needed to provide information on the effects of vaccine formulations on mucosal immunity so that we can formulate any potential antigens into a vaccine that induces the responses identified as protective by (1); thus integrating the 3 approaches needed for optimising vaccine success.

Developing a staged approach to GIN vaccines (Section 7):

As mentioned above, in the final section of the review we present a framework for exploiting existing knowledge in short-term studies towards vaccine development and also a proposed framework for the full rational selection of novel vaccines based on the principles of reverse

vaccinology as outlined above. To develop and maintain the capacity and capability for such a process, longer-term funding is crucial for "research careers in parasitology" outside of simply "academic lectureships". The development of the *Rhipicephalus (microplus) australis* vaccine required a decade of intensive research, and deliberately targeting scour worms would need a similar time-frame.

Abbreviations:

AH, alhydrogel/ aluminium hydroxide
APY, apyrase
ASBV, Australian Sheep Breeding Value
CarLA, carbohydrate larval antigen
CC, chemokine
CCR, chemokine receptor
CD4+, T-helper cells expressing CD4 marker
Ct, cholera toxin
DC, dendritic cell
DXS, dextran sulphate;
ES, excretory-secretory
EST, expressed sequence tag
EV, extracellular vesicle
FC, follicular cell
FCA/ IFA, complete/incomplete Freunds adjuvant
FWEC, faecal worm egg count
GIN, gastrointestinal nematode(s)
Hc, *Haemonchus contortus*
ICM, innate lymphoid cell
IFN-, interferon
IL-, interleukin
IM, intramuscular
IP, intraperitoneal
IPM, integrated parasite management
JPP/IPP, ileal/jejunal Peyers patches
L3/L4, third/fourth stage GIN larvae
Mab, monoclonal antibody
MALT, mucosal-associated lymphoid tissue
MDP, muramyl di-peptide
miRNA, microRNAs
MLs, macrocyclic lactones
MMC, mucosal mast cells
PLT, peptidyl-leukotrienes
PPMO, phosphorodiamidate morpholino oligomers
PPR, pattern recognition receptors
PPRI, peri-parturient relaxation of immunity
SC, subcutaneous
SSH, suppressive-subtractive hybridisation
Tc*, Trichostrongylus colubriformis*
Telc, *Teladorsagia circumcincta*
Th2, T-helper cell type 2
TLR, Toll-like receptor
TNF, tumour necrosis factor

Table of contents

1 Introduction

GIN parasites cost the Australian sheep and goat industries around AUD 450M pa (Lane et al., 2015), encompassing lost production and control measures and amounting to around 8% of the value of the Industry. With the distribution of small ruminant enterprises located in the subtropical and temperate climatic zones in Australia (https://www.mla.com.au/prices-markets/Trends-analysis/livestock-distribution-maps/), a significant proportion of this cost is attributable to the combined impact of several species of "scour worms", including the abomasal parasites *Teladorsagia (Tel) circumcincta* ("small brown stomach worm") and *Trichostrongylus (T) axei* ("stomach hair worm"), small intestinal GIN, *T.colubriformis* and *T. vitrinus* ("black scour worms"). These two genera are the most important scour worms in terms of parasitism and economic impact, and widespread anthelmintic resistance is a particular impediment to their efficient control. Nematodes of lesser significance include *Nematodirus* spp ("Thin-necked intestinal worm"), *Cooperia* spp. ("intestinal hair worms"), and the large intestinal nematodes *Chabertia (C) ovina* ("large-mouth bowel worm") and *Oesophagostomum* spp ("large bowel worms") (Table 1).

The barbers pole worm, *Haemonchus contortus*, is considered the most serious nematode of small ruminants on a global basis (Besier et al 2016; Emery et al., 2016), and is the dominant nematode in summer rainfall and some uniform rainfall regions of Australia, and of sporadic importance elsewhere. However, since around 60% of the sheep industry is located in temperate "winter rainfall" zones, this review specifically addresses the priority "scour worms", and *H.contortus* is not considered in detail other than discuss the development of a vaccine against it.

Table 1: "Scour worms" and their characteristics

Species	**Common name**	**Location**	***Egg production**	****Rainfall zone**	
				Summer	**Winter**
Tel.circumcincta	Small brown stomach worm	Abomasum	10-350	++	+++
T.axei	Stomach hair worm	Abomasum	100-200	++	+++
T.colubriformis	Black scour worm	Small intestine	250	+++	+++
T.vitrinus	Black scour worm	Small intestine	250	+	+++
Nematodirus spp	Thin-necked intestinal worm	Small intestine	50	+	++
C.ovina	Large mouthed bowel worm	Large intestine	unknown	+	+++

* Daily egg production per female worm

** Data from Wormfax 2015-7 (https://www.dpi.nsw.gov.au/about-us/publications/wormfax) and other sources.

Under continuous grazing systems, pasture availability of GIN ("y axis") follows a generic seasonal pattern depicted in Fig. 1. Rainfall and temperature are the principal determinants of L3 availability and survival, with the greatest nematode risk where in environments where relatively mild conditions prevail for extended periods of the year (Levine 1963; Anderson et al 1978; O'Connor et al 2006).

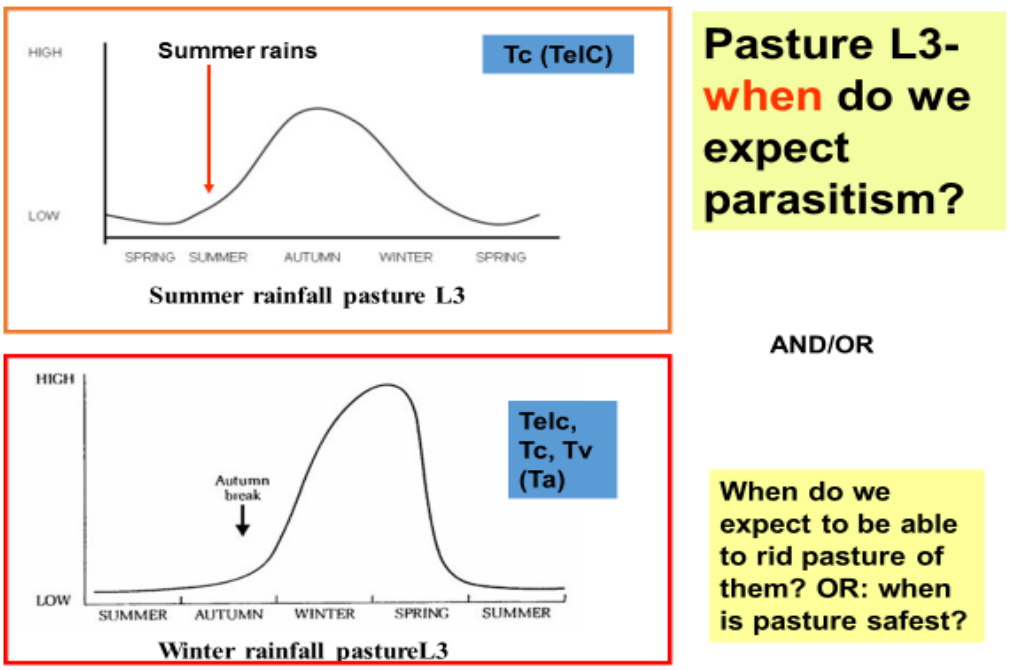

Fig. 1: A "generalised" epidemiological schematic for the seasonal levels of infective GIN L3 on pasture. Data is based on samples from pastures continuously grazed during the 1970s (Armidale and Victoria).

1.1 Biology and pathophysiology of the major scour worms

The most important GIN parasites in winter rainfall zones are *Tel. circumcinta* and *T. axei* in the abomasum, and *T. colubriformis* and *T. vitrinus* in the small intestine, as well as several *Nematodirus* species (Grillo et al., 2008). In summer rainfall zones, the most important worms are *H.contortus, O. columbianum* and *T. colubriformis* (Donald et al., 1978).

***Teladorsagia* (previously *Ostertagia*) *circumcincta* or the brown stomach worm** parasitises sheep, as well as goats and camelids. (*T.davtiani* and *T.trifurcata* are probably phenotypic variants (morphotypes) of *Tel c;* Grillo et al., 2008; Riggio et al., 2013). It is especially common in winter rainfall and cool temperate areas, and is responsible for large economic losses (Nieuwhof & Bishop 2005; Lane et al., 2015; Morgan et al., 2013).

The life cycle is relatively simple, and common to all "strongyle" nematodes (superfamily Trichostrongyloidea (Emery & Beveridge, 2015). Male and female adults breed on the abomasal surface. Eggs are passed in the faeces, where they develop through the first (L1), second (L2) and third (L3) larval stages. The third stage larvae, which is the only stage infective to the host, move from the faecal mass onto pasture, where they may be ingested by grazing animals. Following ingestion, these larvae rapidly moult within 48 hours into fourth stage larvae (L4) which develop in the gastric glands. About 8 days after infection, the young adults emerge from the gastric glands, mature and breed. The prepatent period has been reported to be as short as 12 days (Stear et al., 1995), but in field situations it usually takes 16 days or longer. The number of eggs produced by mature females is heavily dependent on the host immune response (Stear & Bishop, 1999), with an output around 100-200 eggs per day.

The pathophysiology of *Teladorsagia* infection is complex. After ingestion of L3, development to the L5 stage in the gastric glands (akin to *Ostertagia* in cattle) leads to pressure necrosis in the abomasal mucosa and extensive damage to parietal cells, in turn causing a decrease in hydrochloric acid production, elevated plasma pepsinogen levels, reduced protein digestion and microbial control (McKellar et al 1993; Simpson, 2000). However, the immune response itself appears to be responsible for much of the pathology following nematode infection (Stear et al., 2003) as experimentally, immune suppression abolishes many of the clinical signs (Greer et al., 2005). Infection causes a relative protein deficiency that leads to reduced weight gain or even weight loss, due in significant part to a reduction in appetite (Stear et al., 2000; Wallace et al., 1998). There is also a loss in digestive efficiency: lesions in the epithelial barrier allow a loss of protein and in addition protein is diverted to tissue repair and immune and inflammatory processes. Protein supplementation of the diet can prevent the appearance of clinical signs (Stear et al., 2000; Coop & Kyriazakis, 1999) which argues strongly that pathogenesis is a consequence of the relative protein deficiency. Many of the effects such as epithelial hyperplasia, increased pH in the abomasum, pepsinogenaemia and anorexia are a consequence of repair processes triggered by the binding of epithelial growth factor in saliva to receptors on the internal surface of epithelial cells (Stear et al., 2003). Prolonged and sustained infection subverts the repair process into a pathogenic mechanism.

Protective immunity to *T.circumcincta* develops after continual exposure to the parasite over a prolonged period (Seaton et al., 1989); however, the degree of immunity acquired is dependent on the level of parasite challenge, age of the animal and its genotype (Singleton et al., 2011). In practice, immunity usually develops too late to prevent the parasite's negative effects on lamb productivity, unless an effective control program is in place. Adult sheep in sound nutritional condition exhibit a strong resistance to infection, and female worms that do develop produce relatively few eggs.

Trichstrongylus, or the black scour worm comprises several species found in sheep and goats, some of which also occur in cattle and other host species. The most significant in Australia are the small intestinal species *T.colubriformis* (common in most sheep-grazing environments) and *T.vitrinus* (mostly winter rainfall regions), with the less-pathogenic *T.rugatus* is largely confined to drier inland areas (de Chaneet and Dunsmore 1988, Beveridge et al 1989a). The abomasal species *T.axei* is more commonly a parasite of cattle, but significant infections of sheep are occasionally seen. Outside the host, the life cycle of all is similar to that of *T.circumcincta*, with an interval of between infection by the L3 stage and egg production by female worms (the pre-patent period) of nearly 3 weeks. Within the host animal, exsheathed L3s of the intestinal species burrow between the enterocytes of the villi for 10-14 days, causing the development of intra-epithelial tunnels and erosion and flattening of villi in the anterior 5m of the small intestine (Barker 1975; Beveridge et al 1989b). This causes signs of generalised enteritis, including haemorrhage, hypoalbuminaemia and hypoproteinaemia with fluid loss (Barker & Titchen, 1982), and consequent impairment of digestive functions (Coop & Kyriazakis 1999).

Other "scour worms" of significance in Australia include several species each of the small intestinal genera *Nematodirus* and *Cooperia*, both relatively common throughout sheep grazing regions (Donald et al 1978; Besier, 2004). *Nematodirus* is considered the more pathogenic, especially in lambs, although damaging infections occur only sporadically. The large intestinal nematode *Chabertia* ovina, common in winter rainfall regions is also relatively pathogenic but is rarely the

major species responsible for clinical disease outbreaks. *Oesophagostomum columbianum*, also found in the large intestine, was once a significant cause of loss in summer rainfall regions of Australia, but is highly susceptible to anthelmintics and is no longer common, and the sister species O*.venulosum* is considered of relatively low pathogenicity (Besier, 2004).

The clinical signs of GI "scour worm" nematodes are similar for all species of importance in Australia, and disease occurrences are usually the result of mixed-species infections in sheep (reviewed in Levine 1980, Urquhart et al 1996; Emery & Beveridge, 2015). Diarrhoea (scouring) is the classic sign in acute cases, with the important secondary effect of an increased risk of breech strike by blowflies attracted by faecal soiling (dags). Deaths may occur if severe infections are left untreated, especially in lambs. However, the major cause of loss to the sheep industry results from the effects on production: ill-thrift, poor weight gains, loss of weight and body condition, and reduced wool production.

Acute nematode infections are most commonly seen in lambs in the first months of life, but significant effects are common until a functional immunity to nematodes develops, usually between 8 and 15 months of age, depending on the timing and extent of larval exposure. In mature animals, clinically significant signs are seen mostly in ewes experiencing the peri-parturient relaxation of resistance (Salisbury and Arundel 1970; Beasley et al 2010) and animals in poor nutritional condition, in which the production effects are exacerbated by worm infections.

As a variant to the classical expression of nematode infections in susceptible host animals, a hypersensitivity syndrome has been recognized as an additional cause of transient diarrhoea sheep. This is seen in previously worm-exposed flocks, in which the ingestion of nematode L3 incites an immunological and gut inflammatory response which prevents the development of the larvae to adult worms, but results in scouring in a variable proportion of the flock (Larsen et al 1999; Jacobsen et al 2009; Williams & Palmer, 2012). Scouring may occur with exposure to even relatively low numbers of L3 (Larsen & Anderson 2000). While faecal worm egg counts typically remain low and anthelmintic treatment is ineffective, the scouring resolves spontaneously within a few days in individuals, although it may take some weeks before all sheep return to a normal condition. Interestingly, the syndrome occurs differently between locations: in temperate Victorian environments, it is relatively common in young ewes in winter and spring (Larsen et al 1995), whereas in the Mediterranean climate of Western Australia, outbreaks (also in Autumn and Spring) occur only sporadically, in sheep from 18 months onward (Jacobsen et al., 2009). However, despite its significance as a cause of soiled wool and predisposition to blowfly strike (Larsen et al., 1995), the development of preventative measures awaits further research.

1.2 The epidemiology of nematode infections

The development of efficient nematode management programs is based on an understanding of the prevalence, severity and seasonal occurrence of nematode infections in sheep, which depends chiefly on the ecology of their free-living stages on pasture, and the timing and effectiveness of the natural immunity of host animals. Although the basic ecological requirements are similar for the major scour worms in sheep in Australia, between-species differences within the genus *Trichostrongylus* allows it to exist across a broad climatic range (Callinan 1978, Callinan 1979; de Chaneet & Dunsmore 1988; Beveridge et al 1989a, O'Connor et al 2006), whereas *T.circumcincta* is better adapted to cooler environmental conditions (Gibson & Everett 1972; Callinan, 1978; Young

1983; O'Connor et al., 2006). For worm control programs developed for a particular environment, however, strategic treatments and management interventions aim to encompass both these genera and any endemic minor scour worm species, although separate control programs are typically needed for *H.contortus* (Besier, 2004).

In general, the abundance and annual distribution of rainfall is the primary determinant of the endemic range and seasonality of trichostrongyloid nematodes (Levine 1963; Anderson et al., 1978), and the presence of green pasture growth, as an indicator of moisture availability at ground level, provides a proximate indication of the favourability for development of the eggs through the larval stages, and migration of L3s onto pasture (Besier, 2004). Once moisture requirements are sufficient to permit egg and larval development, the rate of larval development is largely temperature-driven (O'Connor et al., 2006). High summer temperatures are a critical restriction on the development of worm eggs and the survival of the larvae, especially in winter rainfall regions, but while low temperatures in winter may lead to a temporary (and usually intermittent) cessation in the development of worm eggs, there are few locations in Australia where they are sufficiently low to significantly inhibit the survival of scour worm L3 on the pasture.

An understanding of the period for which pastures are infective to grazing livestock in particular environments is a key element in the development of strategic preventative programs, as the L3 stage is relatively tolerant of conditions inimical to the earlier stages (Levine 1963; Brunsdon 1980). Populations of L3 can remain viable on pasture for many months, but this varies greatly between seasons and environments. In winter rainfall regions, a proportion of L3 present on pasture in autumn typically survive (in declining numbers) until late spring, whereas the majority of L3 which develop and migrate onto pasture in spring die quickly once hot summer temperatures occur.

Several mechanisms explain the survival of nematode populations through hot and variably dry summer conditions (other than as adult worms in animals not treated in summer):

- the survival of some L3 in the herbage of perennial plant species pastures, where there is sufficient summer rainfall (or, more rarely, irrigated pastures);

- the survival in the faecal pellet of L3 that develop to this stage in spring but fail to migrate due to dry external conditions; some of these then emerge onto pastures some months later and are available to grazing livestock in autumn (Young & Anderson, 1983, Besier & Lyon 1990).

- Hypobiosis of fourth stage larvae: infective L3s of *T.circumcincta* ingested during spring may undergo hypobiosis (Gibbs, 1986) at the early L4 stage in early summer and resume their development in the following early autumn, somehow sensing the "autumn break". Hypobiosis appears to occur under the harsh summer conditions of inland Western Australia (Woodgate et al 2011), although this requires confirmation, and whether it also applies to Trichostrongylus or in other environments within Australia, has not been specifically tested. There is no evidence for hypobiosis as a winter survival strategy in scour worm nematodes in Australia, although it is has been demonstrated to occur in *H.contortus* (Anderson et al., 1978; Donald et al., 1978b).

Temperate winter rainfall regions of Australia (Victoria, SE South Australia, Tasmania, southern NSW):

The development nematode eggs to L3 occurs reliably from early-mid autumn, when large numbers of L3 become available on pasture following the onset of rain (Figure 1), and continues until late spring, when L3 populations decline ((Anderson et al., 1978) . Pasture contamination with worm eggs in autumn is critical to the development of significant worm burdens in sheep in winter (Anderson, 1972; 1973), which may lead to clinical helminthosis. Depending on when they are born, lambs are exposed to L3 which emerge from faecal pellets or have survived on over summer pasture, and also L3 developing as a result of the increased worm egg deposition due to the peri-parturient relaxation of resistance by the ewes. Worm burdens in lambs are typically significant by early summer and their removal is necessary both to prevent disease and to limit over summer worm survival. However, autumn and winter-born lambs generally receive sufficient exposure to infection to develop a functional natural immunity to worm infection by summer and do not re-acquire heavy worm burdens after treatment. Spring-born lambs may remain relatively worm-susceptible until the following autumn or winter, depending on when sufficient L3 intake occurs to provoke immune development.

While sheep are generally functionally resistant to infection by 12 – 18 months of age, and significant helminthosis is uncommon in well-nourished mature sheep, clinical disease outbreaks can occur in ewes during lactation if they encounter large L3 populations prior to lambing. Sub-clinical worm infection is a significant cause of reduced sheep production in sheep of all ages and is exacerbated under poor nutritional conditions.

Mediterranean climatic regions (SW Western Australia, parts of South Australia)

Although the general seasonal pattern is similar to that in the more temperate regions in the winter rainfall zone, nematode epidemiology is dominated by the almost complete cessation of L3 intake for 3-4 months (or longer) during summer and early autumn, and a consequent short period favourable for larval development, except in milder south-coastal environments (Besier & Lyon 1990; Besier, 2004).

Poor L3 survival in summer favours worm control, as effective summer anthelmintic treatments successfully prevent significant autumn pasture contamination with L3, but despite this, the survival of relatively small worm populations over summer (in sheep, or in faecal pellets on pasture) commonly leads to helminthosis in susceptible sheep in winter and spring. As cereal cropping is the dominant agricultural enterprise in most of this environment, a large proportion of most farms is unavailable for grazing for most of the period of pasture growth (autumn to early summer), and the increased winter stocking rates of sheep on the remaining area increases L3 intake rates.

Summer rainfall zones (Northern NSW, Queensland)

Although *H.contortus* is the dominant nematode, *Trichostrongylus* species (and to lesser extent, other trichostrongyles) are a significant cause of reduced sheep production and occasional disease, especially in lambs and weaner sheep (Anderson et al 1978, Bailey et al 2009a). Conditions for the development of worm eggs to L3 become especially favourable in early summer, shortly after lambs are born in spring, and uncontrolled burdens increase from then on to peak in late winter before immunity develops, at around 12 months of age (Anderson et al.,1978). In areas of high altitude (the Northern Tablelands), very cold winter temperatures reduce or prevent nematode egg development

for 2-3 months, which can be used as a pasture management strategy for worm control (Bailey et al 2009b).

In dry inland regions, where conditions favourable for L3 development are relatively shorter and less reliably seasonal than in temperate-climate areas, and properties are sparsely stocked, nematode infections are considerably less severe and clinical helminthosis more sporadic. However, after periods of prolonged rainfall and pasture growth, outbreaks of haemonchosis with significant sheep losses can occur, especially on large pastoral properties where sheep are only occasionally observed or mustered. These events are reasonably predictable on the basis of rainfall and pasture growth, but this is often in hindsight.

1.3 Management of sheep nematodes in Australia

The potential for developing efficient and effective annual worm control programs was recognised some decades ago, with studies of the epidemiology of helminthosis in relation to climatic and seasonal conditions (Barger, 1999), and the demonstration of significant losses due to poorly-managed worm infections. The conceptual aim of preventing pasture contamination with worm eggs at critical times to minimise the development of damaging worm burdens in sheep was translated into "strategic" programs specific to particular environments, exemplified by the "summer drenching" program which continues as the basis of effective control in all winter rainfall regions (Anderson 1976; Morris et al 1977).

From the outset, the programs developed to manage both helminthosis and anthelmintic resistance were based largely on integrated parasite management (IPM) principles (Brunsdon 1980; Waller 1999), including pasture management to minimise L3 intake, the informed use of anthelmintics and a recognition of the key role of natural immunity to nematodes. These programs were later extended to include measures intended to reduce the development and impact of anthelmintic resistance, and later the routine monitoring of faecal worm egg counts, and promoted to the sheep industries as regionally and target worm-specific programs (Besier 1997), including "WormKill" (NSW) and "Wormbuster" (Queensland) for *H.contortus* in summer rainfall zones; "Drenchplan" (NSW), "Wormplan" (Victoria), "Wormwise" (WA) and Drenchcheck (SA) in winter rainfall regions; with modifications for uniform rainfall and inland pastoral regions. These programs received general industry support and were promoted through strong multi-institution extension campaigns, with adoption of the major elements by many sheep producers (Besier & Love 2003).

More recently, the increasing severity of anthelmintic resistance and necessarily increased complexity of "sustainable" worm management recommendations led to the development of the WormBoss program (Le Feuvre et al 2005; Woodgate et al 2006; www.wormboss.com.au), providing online information on nematode biology, treatment strategies and anthelmintics, and regionally-appropriate control guidelines. The larger entity, ParaBoss, (Besier and Kahn 2015; www.paraboss.com.au) continues to develop as the national authority for ruminant parasite management, with new technologies, including vaccination against nematodes (eg, Barbervax; Besier et al., 2015)) and biological control (e.g., Bioworma; Healey et al 2018) incorporated into recommendations for planning efficient and sustainable parasite control.

Nematode management programs

The basic elements of nematode management recommended in ParaBoss programs, into which new nematode vaccines would be incorporated, are based on a "prevention-detection-response" approach.

Prevention includes:

- Strategic control programs: these specify the times of routine annual treatments (anthelmintic or pasture movement) most effective for minimising L3 development and intake, mostly as developed many years ago but more recently modified through the advent of computer simulation modelling to optimise management interventions (Barnes et al 1995; Dobson et al 2011).

- Pasture management policies, whereby sheep considered especially worm-susceptible are grazed on pastures known to be relatively "worm safe" by animal management (pasture spelling) or specific practices (e.g., grazing after cattle or fodder crops; Barger, 1999). Key to their success is an understanding of the likely L3 infection risk, based on ecological studies for particular environments (see http://www.wormboss.com.au/news/wormboss-online-learning.php).

- Genetic selection for natural resistance or resilience to worm infection: This is now well-established in breeding programs, with worm resistance integral to multi-trait selection indices in online ram comparisons provided as Australian Standard Breeding Values (ASBVs, Woolaston & Windon 2001; Kelly et al 2013). Despite the importance to commercial producers, there are few rams with authenticated ASBVs for FWEC, which requires worm exposure of progeny for evaluation. This likely reflects the reluctance of breeders to compromise ASBVs for productivity traits of progeny by GIN infection from pasture or other means.

- Nutritional management: the pivotal role of nutrition in maximising the resistance and resilience of sheep to worm infections has long been recognised (e.g. Coop & Kyriazakis, 1999; McClure & Emery 2007)), and is especially important in Australia where large seasonal variations in pasture quality and quantity are usual in most sheep grazing regions. Relatively recently, it has been recognised that the recommendations for optimal sheep production, especially for Merinos, promoted to industry in extension programs such as "Lifetime Ewe" and "Making More from Sheep" (http://www.makingmorefromsheep.com.au/), generally ensure that nutrition is adequate to maintain immunity to worm infection. The increasing adoption of body condition scoring at intervals as a management tool for reproductive and production purposes will assist in minimising the impact of nematodes due to poor nutrition, as the use of condition scores to gauge resilience to worms has been demonstrated in studies to develop targeted selective treatment recommendations (Besier et al 2012; Cornelius et al 2016).

- Vaccination: the introduction in 2014 of "Barbervax" (https://www.agric.wa.gov.au/livestock-research-development/development-and-production-barbervax-vaccine-against-barbers-pole ; Besier et al., 2015) provides a novel

alternative to anthelmintic treatment, through the "epidemiological effect" of a reduction in pasture contamination with *H.contortus* eggs (Le Jambre et al, 2008). Despite limited uptake to this point, believed partly due to the requirement for multiple vaccinations at relatively short intervals, supply problems and possibly the cost, the adoption of vaccination continues to increase in regions where few anthelmintics remain effective against *H.contortus*.

- Biological control: the potential for the practicable deployment of nematophagous fungi long the subject of research interest (Waller *et al*., 2006), has recently been realised in the commercial availability of "Bioworma". Provided over a prolonged period as a feed supplement to grazing animals, this results in an epidemiological effect similar to that of against Barbervax, but encompasses all worm genera. However, the need for continuous administration is likely to limit its use in extensively-grazed sheep. Other biological control concepts, such as the use of pastures with anthelmintic properties (Hoste et al 2006), have shown potential but have not yet been developed into practical approaches.

Detection

Formerly confined largely to diagnosis or research, over the last 2 decades the periodic monitoring of faecal worm egg counts (FWEC) has become a routine part of worm management programs in Australia. The chief purpose in control programs is to alert to impending worm disease or sub-clinical production loss, and to indicate whether anthelmintic treatments usually given at a particular time are in fact necessary. Recommendations for monitoring schedules vary, with more frequent monitoring appropriate for more pathogenic nematodes (*H.contortus*) and in regions and seasons where parasite challenge is especially high. Within vaccination-based worm management programs (as with Barbervax), where the total removal of worm burdens is not expected, WEC are an important precaution to confirm effectiveness or indicate failure. However, this has not been widely practised (Reeve & Walkden-Brown, 2014). FWEC services are widely available, and a proposed Quality Assurance evaluation for FWEC proficiency is under development through the "ParaBoss" program.

Response

The action appropriate when significant nematode burdens are detected may involve flock movements to prevent the intake of large numbers of L3, but more commonly involves the use of anthelmintics. The selection of an appropriate product (active ingredient; narrow or broad-spectrum) and formulation (short or long-acting) is essential for effective (and sustainable) treatment, and the use of inappropriate products is a common explanation of control failures or inefficient treatments (Besier et al 2012).

However, the major requirement for appropriate decisions on anthelmintic choice is the status of resistance by the nematode species on a particular property (reviewed below). Testing of available anthelmintics has long been a cornerstone of drench resistance management (Prichard et al 1980), but the number of tests conducted has been falling for some years. Drench testing is now practiced as a routine by very few sheep producers, and wider uptake probably awaits the development of practical *in vitro* tests (at present, apparently a distant prospect for the anthelmintic groups still likely to be effective in Australia).

Anthelmintics and anthelmintic resistance

Australian sheep producers have access to a wide range of anthelmintic actives, but resistance is present to all and common to most, by at least some of the common nematodes (Besier & Love,2003; Playford et al 2016). ("Resistance" is defined for this purpose as producing a reduction in worm egg count of less than 95% for any one worm genus at the label-recommended dosage.) Given that few sheep producers are aware of the situation in their flock, a significant on-going loss of production related to worm infections must be expected. The availability of a vaccine to minimise scour worm challenge would reduce dependence on anthelmintics, both reducing the penalty imposed where treatments are only partially-effective, and assisting to delay the further development of resistance.

With an increase in the severity of resistance, products containing a combination of anthelmintics were introduced to maximise efficacy, and more recently these formulations have been specifically advocated as a strategy to reduce the rate of development of resistance (Leathwick & Besier, 2014); at present, combination products account for close to half of all anthelmintic sales in Australia. A number of narrow-spectrum anthelmintics are available (some also providing activity against the liver fluke), and some long-acting products are available as injections or slow-release capsules.

Recent information regarding the prevalence of resistance is lacking, as the number of tests completed each year is low (probably well less than 100 annually, across all states) and testing varies in intensity between regions, and results are variable in quality (the range of anthelmintics tested varies, and many tests do not include results differentiated to nematode genus). However, based on the most recently-published data (Playford et al 2014; "WormMail", pers. com., Dr S. Love NSW Dept. Primary Industries), and occasional reports at conferences or from laboratory sources (B.Besier, pers. com.), an approximation can be made of the likely situation in Australia:

<u>Broad-spectrum, short-acting single-actives</u>:

Benzimidazoles (BZs): Resistance by most major genera is present on the great majority of properties, and in practice there are no situations where this group would now be recommended as a single-active drench.

Levamisole (LV): Resistance by the scour worm genera is present on the great majority of properties, especially in the winter rainfall regions, and it also has little role as a single-active in that situation. Against *H.contortus*, resistance is highly prevalent (70% or more of properties) in summer rainfall regions, but it remains relatively effective in regions where this species is only an occasional threat.

Macrocyclic lactones (MLs): There is wide variation between the available actives, with resistance to the first-introduced ivermectin widespread, and given the greater potency of the other MLs (Leathwick et al., 2000) its use can no longer be justified. Resistance to the more potent abamectin has increased markedly in recent years, and is present against either or both the genera *Haemonchus* and *Teladorsagia* on at least 70% of properties. Although it is still useful in combination products, without testing for ML resistance it is difficult to justify the continued use of abamectin alone. However, it appears that the most potent ML, moxidectin, remains effective on the majority of properties (at present probably 30% or more, but decreasing) presumably as a reflection of the

greater “head kill” (LeJambre et al., 1999), despite its position as the largest-volume single-active sold in Australia.

Monepantel: Although introduced to Australia some 7 years ago, the use of monepantel remains low (understood to be ~6% of sales), presumably because the cost to producers is several times that of other drenches. Despite this, resistance has been confirmed on at least 3 properties in Australia (Dr S. Love, pers. com.), and is suspected on others. As with reports from other countries, each report of resistance has involved several genera, and efficacy has declined relatively more rapidly than is usual for other anthelmintics (Scott et al., 2013).

Broad-spectrum, short-acting combination

Benzimidazole-levamisole: While this combination remains effective on a surprising number of properties (~10%), in practice it has been overtaken by the “triple combinations” and has a small and declining market.

ML combinations: Combinations of abamectin, a benzimidazole and levamisole are now widely used, although to this point resistance appears to occur on less than 10% of properties, with efficacy generally still >90% where resistance has been reported. (A combination of abamectin-benzimidazole-naphthalophos is also available.) The most recently-introduced in this group is a moxidectin-benzimidazole-levamisole combination, expected to be the most potent of the type, and to which no resistance has yet been reported.

Derqantel-abamectin: The sole member (“Startect”) became available only a few years ago and has a limited sales volume (presumed due partly to price, next highest to monepantel). Despite anecdotal reports of reduced efficacy, there appear to be no confirmed reports of resistance in the scour worms.

Monepantel-abamectin: This combination was introduced very recently, presumably in response to reports of monepantel resistance, and at present has a minimal sales volume.

Narrow-spectrum, single actives:

Naphthalophos: Although fully effective for *H.contortus*, naphthalophos was marketed as a “mid-spectrum” product for scour worm control, but is no longer generally available. Almost all use now is in combination with abamectin and levamisole; this has a limited sales volume, but is presumed to be at least as effective as the abamectin-benzimidazole-levamisole products.

Closantel: This long-acting flukicide/anthelmintic, a halogenated salicylanalide, is specific to *H.contortus* amongst GIN, in which resistance is common in the major endemic zones, although it remains highly effective in other situations.

Long-acting products

Prolonged anthelmintic activity is available – at a considerable cost – from several products, with significant usage, mostly as pre-lambing treatments to ewes. These include a slow release capsule formulation, a product releasing abamectin and albendazole continuously over 3 months (capsules

that contain older actives can no longer be recommended), and is active against susceptible worms of most major species. A moxidectin depot injection is also available, releasing active for a slightly shorter period for *Teladorsagia* (and *Haemonchus*) than the capsules, and for half that period for *Trichostrongylus*.

Although reported to provide highly-effective worm suppression, there is a markedly higher risk of selection for resistance than for short-acting anthelmintic (Le Jambre et al 1999), and long-acting products can be recommended only according to specific drench resistance management strategies (although in practice, they are usually used without such consideration).

Anthelmintic resistance – species involved and the implications

The inefficiencies of worm control imposed by anthelmintic resistance significantly increase the necessity for non-drench-based alternatives, including vaccination. However, although resistance to the benzimidazoles, levamisole and monepantel has been demonstrated in several nematode genera, it should be noted that confirmed resistance to the MLs principally involves only two main genera: *Haemonchus* and *Teladorsagia*. In Australia, this translates to serious resistance to the MLs in *Teladorsagia* in winter rainfall regions, and in *H.contortus* in summer rainfall areas.

While reports of resistance in *Trichostrongylus* spp. (and some other species) exist, few instances have been confirmed by total worm counts (and these mostly in goats). This presumably reflects the relatively poor genus-specificity of worm egg count-based drench resistance tests, which usually rely on the commonly-used larval culture and differentiation technique, which does not distinguish precisely between *Teladorsagia* and *Trichostrongylus* (Roeber & Kahn 2014). Few or no cases of ML resistance in *Nematodirus* have been reported, although if resistance was common it should be evident, as genus identification is based on egg morphology.

However, the implication that the great majority of anthelmintic treatments in Australia will generally be adequate against *Trichostrongylus* because they include an ML anthelmintic, and that alternative approaches therefore have a limited role, is incorrect:

- In almost all cases, in the major areas endemic for the scour worm species (winter rainfall regions), the worm populations are multi-genus, due to generally similar ecological requirements. It is usual (in Australia) that *Teladorsagia* and *Trichostrongylus* occur simultaneously (albeit in variable proportions), with a range of less-important genera (*Nematodirus*, *Chabertia*, *Oesophagostomum*, *Cooperia*).

- Although situations occasionally occur where *Trichostrongylus* and other genera are the major part of a worm population in a winter rainfall region, and *Teladorsagia* is relatively insignificant, this is rarely known before treatment because genus identification requires laboratory tests. Anthelmintics given “blind” will commonly fail against a proportion of the population. (Note: this is less usual in summer rainfall regions where *H.contortus* is the dominant species for most of the year, and periods when other species are likely to be significant are more easily predicted.)

- Regardless of anthelmintic efficacy, worm control programs based only on drenching are rarely optimally efficient (unless treatments are given at an unsustainable frequency). Periods of excessive L3 intake often occur, but can be avoided through non-chemical measures with the epidemiological effect of preventing heavy pasture contamination with worm eggs – this is a key intent for vaccination programs.

- Although resistance to MLs by *Trichostrongylus* and the minor genera may not be common at present, it has been demonstrated to occur. With the heavy and increasing reliance on ML-based products, there is a reasonable likelihood that it will eventually develop to a significant degree.

- *Trichostrongylus* is especially ubiquitous as a genus, and as anthelmintic-based control fails, a vaccine against it would have wider use than one for the *Teladorsagia*, which is largely a problem of winter rainfall regions.

Nevertheless, although vaccines against *Trichostrongylus* and possibly some minor genera (and improved vaccines for *H.contortus*) will have a role, widespread ML resistance suggests a particular priority for a vaccine against *T.circumcincta*.

Anthelmintic resistance management

Strategies to minimise the development and impact of resistance to anthelmintics are integral to efficient control programs, and have long included recommendations for the use of non-chemical approaches (Waller, 1999). However, in practice, "resistance management" strategies (detailed in the WormBoss website), presently centres mostly on changes to modes of anthelmintic use, including ensuring that the appropriate dose is given, minimising the frequency of treatment, confining long-acting treatments to appropriate situations, and avoiding the introduction of resistant worm populations.

However, the uptake of even these basic recommendations has been variable and far from universal (http://www.wormboss.com.au/news/articles/general/national-survey-highlights-parasite-control-practices.php). More complex strategies, including the application of the "refugia" concept (the planned retention of non-resistance worms, to dilute resistant populations; (Besier 2012; Leathwick & Besier 2014), and the use of pasture management and genetic selection policies to reduce L3 intake, have seen considerably less implementation. Of major concern to industry advisers, the recommendation to ensure an objective basis regarding likely drench efficacy, through resistance testing or by consulting an informed adviser, has been poorly adopted (Reeve & Walkden-Brown, 2014). As a whole, it must be expected that the loss to parasites from the use of products that are poorly or partially effective will continue to increase.

In this context, nematode vaccines would provide a particular benefit, as the property-wide epidemiological benefit of minimising L3 intake, which increases within a season and potentially through successive years, will partly offset the failure to implement other resistance-avoidance strategies and will enhance their effects, even where anthelmintics may not be completely effective. Although vaccination programs require informed management of worm populations, providing these as a package of advice (as required for Barbervax), may find greater acceptance than has occurred for the present suite of recommendations.

1.4 Scope of the Review

Vaccines have a long history of success for the control of infectious diseases, utilising the characteristics of the host's own protective immune responsiveness to prevent and control pathology and production loss. Since successful vaccination against GIN requires an effective mucosal immune response, the predominant delivery of antigens or organisms in livestock has been systemic injection or oral delivery. The recent success and efficacy of Barbervax ™, which achieves around 80% protection against Barber's pole worm (*Haemonchus* -Hc) through the generation of high titres of antibody by 6 inocula of native gut antigen (H11) over 18 months (Le Jambre, et al., 2008.), raises the possibility for vaccination against other GIN. To this end, this review has 4 aims;

1. to review past and current literature on scour worm vaccines to provide reasons for the lack of (reproducible) success;
2. to examine new technologies and advances in the 'omics areas which address past limitations and enable more incisive research into the host-parasite interaction, antigen identification, vaccine formulation and delivery
3. to provide an integrative extension approach for the use of vaccines in IPM
4. to outline a process for development of scour-worm vaccines that enables investment towards success.

2 Development of immunity and resistance to Scour Worm GIN in small ruminants

2.1 Cross protection against GIN

Since most GIN infestations are mixed, many reports have identified both physiological and immunological regulation of parasite numbers in preferred niches in the GI tract in small ruminants dosed with single (bolus) or continuous (trickle) doses of infective L3. In sheep necropsied 3 months after dosing orally with 10,000 HcL3, 25,000 TelL3 and 25,000 *T.axei* L3, Turner et al., (1962) found that while the numbers of *Telc* and *T. axei* were unaffected the establishment and survival of *Hc* was reduced by >90%. Similarly, the establishment and fecundity of *Hc* was also reduced more than 50% when trickle infections of 6000 *Telc* L3 and 3000 *Hc*L3 per week were given to lambs for 10-13 weeks; *Telc* numbers were unaffected (Dobson and Barnes, 1995). From a 4-year study of the populations of *Telc* and *Tc* in resistant (R) and susceptible (S) rams, Gruner et al, (2004) concluded that the worm numbers, size and fecundity of *Tc* was more intensely regulated compared with *Telc.* These outcomes mirror results from the Rylington flock in Western Australia (Williams et al., 2010). It was also reported that R ram lambs effected a 25% and 96% reduction in pasture L3 populations of *Telc* and *Tc*, respectively, after 2 years (Gruner et al, 2002), consistent with a more pronounced regulation of *Tc*. More recently, Harrison et al., (2003) reported that while 3-5 repeated larval infections of 14 days duration generated high levels of protection against *Hc* and *T.col*, the procedure was not effective against *Telc*. A similar trial using irradiated *Hc*L3 and *Tc*L3 vaccines could only generate around 30% cross-protection as measured by egg counts (Adams et al., 1989).

2.2 Host-parasite interactions related to immunity

2.2.1 Induction and expression of immunity to scour worms

"**Resistance**" to GIN is usually synonymous with the establishment and operation of innate and acquired immune responses. Resistance has a genetic basis, with a moderate heritability (h^2; see below), enabling selection for reduced FWEC and the assignment of ASBVs for reduced FWEC. For ease of analysis and cost, immunity is most commonly assessed by reductions in the FWEC, accompanied by more lengthy larval cultures (or PCR) from faeces if the proportions of GIN are required. When necropsies are performed, immunity can also be expressed as reductions in total and differential worm counts, where experimentally, worm length and female egg content may also be measured. Other measures to quantify developing immunity include dosing regimes with labelled or drench resistant L3 to assess L3 establishment, or the adoptive transfer of parasites followed by worm counts.

"**Resilience**" for parasites is usually defined as the ability of small ruminants to produce and reproduce in the face of "normal" field challenge with GIN. Resilience has small genetic (h^2 around 0.1) and large "non-genetic" inputs (NZ). It is variously measured by liveweight gain, fleece or meat production or characteristics, and/or increased drenching intervals (Bisset et al., 1996; 2001). The mechanism of resilience is incompletely understood, but is highly dependent on nutrition/ food intake/ feed conversion (FC), as resilient animals tend to perform less well under nutritional stress such as drought (MLA, 2017).

Development and expression of "conventional" immunity. In naïve hosts, the establishment rates for a range of gastrointestinal nematodes range from 40-80% (Dobson et al., 1990). The initial losses are caused by innate inflammatory responses, as rates are enhanced by pre-treatment with corticosteroids (used to maintain donor sheep) (Miller 1996; Emery, 1996). With continuous ingestion of infective L3, small ruminants develop an age-related resistance to worm infestations, with kinetics depicted in Figure 2. The interval of 4-9 weeks for the expression of an escalating level of immunity to *Tc*L3 (Dobson et al., 1990) was similar to that observed when lambs were trickle infected with graded doses of *Hc*L3 (Barger et al., 1985). The seasonal ingestion of new cohorts of *Hc*L3 in Spring elicited a "self-cure" reaction (Stewart, 1955; Blitz and Gibbs, 1972; Adams, 1993) and its "non-specific, collateral damage" also eliminated other abomasal parasites (*Tel.c* and *T.axei*) and downstream GIN such as established *Tc* and *Nematodirus* from the proximal small intestine (Stewart, 1955; Emery et al., 1993; Harrison et al., 1999). Sheep which are fully immune to GIN species and which have been exposed within the previous 7 weeks ('hyper-immune') can mount a 'rapid rejection' response that prevents establishment of *Hc* L3 within 30 minutes (Jackson et al. 1988) and eliminates incoming *Tc* L3 from the entire 15 metres of the small intestine within 2 hours (Wagland et al., 1996). While optimal immunity removed GIN from the entire GIT (Wagland et al.,1996), lesser levels of immunity resulted in establishment further downstream in the small intestine (Harrison et al., 1999). This effect was consistent with the paralysis of GIN motility by the mucosal effector response, which was a reversible inhibition (Emery et al., 1996; Harrison et al., 2008). These kinetics were accelerated in genetically resistant lines of sheep, which developed protective immunity to reduce L3 establishment around 2 weeks earlier than outbred lines when the release of protease from isolated mast cells was assayed during sequential necropsies (Windon, 1996). More rapid induction of anti-L3 responses reduced overall worm burdens and parasitic costs to production

(Windon, 1996). “Resistant” animals may possess immune systems that react quantitatively or qualitatively to lower amounts of worm antigen or have more effective innate immune responses to reduce early infections or activate acquired immunity to reject worms. Alternatively or additionally, they may remove worms with less collateral damage in the gut or have better foraging activity to increase dietary protein, which is essential for optimal development of worm immunity in ovine weaners (Coop et al., 1995; van Houtert et al., 1995) and abrogate the periparturient reduction in immunity (PPRI) (Donaldson et al., 1998). The large component of “environmental “ variation, together with the complexity of the host immune response to differing stages of the parasite and the measurement of FWEC as the phenotype some 3-4 weeks after initial infection, may account for the difficulties with finding reliable genetic and biological markers to allow for genetics strategies to be progressed.

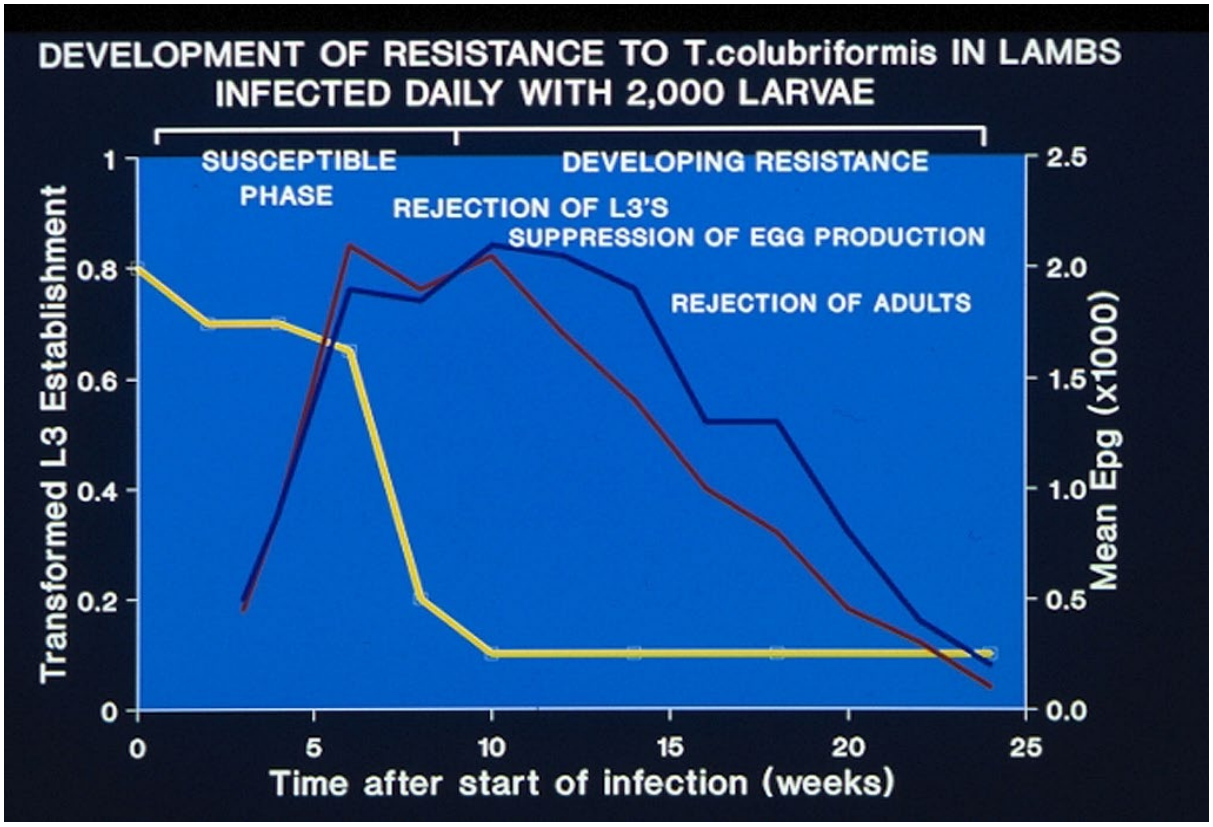

Fig. 2. Kinetics of the development of escalating immunity after infection of lambs dosed with 2000 *Tc* L3 per day (adapted from Dobson et al., 1990). Comparisons include; % L3 establishment (yellow), egg production (as FWEC; red) and worm counts (blue).

At the mucosal interface, the host-parasite interaction and the development of protective immune responses have been analysed through laproscopic biopsy or sequential necropsy (McClure et al., 1992; Rowe et al., 2009) with tissue or contents sampled through indwelling abomasal or intestinal fistulae (Jones & Emery 1994; McNeilly et al 2019; in preparation). These studies indicated that the gross effects of successful immunisation induced as a result of prior parasite exposure effectively removed a challenge infection within 24h, akin to the self-cure reaction incited by HcL3 (McClure et al., 1992), and also demonstrate that the mucosal transcriptome prior to parasite challenge can be predictive of subsequent worm burden (McNeilly et al in preparation).

More recently, an elegant series of studies have examined the development of host mucosal immunity to several GIN through daily monitoring of cellular and proteomic changes in cannulated lymph ducts, continuously draining abomasal or intestinal sites in susceptible and resistant sheep (see Hein et al., 2010). At the local site, the subsequent expression of GIN immunity in ruminants is consistent with a Th2 "allergic" response with production of sensitized CD4+ lymphocytes expressing and secreting interleukins IL5, IL13 and TNFα (Pernthaner et al., 2005; Ingham et al., 2008) and reduced expression of CXCL10 (Andronicos et al., 2010). The Th2 phenotype is evident amongst sensitized cells contained in intestinal and abomasal lymph from immunised-challenged and genetically- resistant sheep (see Hein et al., 2010). With ongoing infestations or infection, ruminant Th1 or Th2 phenotypes become polarized in chronic infections such as bovine tuberculosis, babesiosis (Th1) and liver fluke (Th2; Brown et al., 1998). This accords with the greater flexibility of Th phenotypes (perhaps slower development of effective immunity) during the initial encounters with nematode parasites (Brown et al., 1998; Pernthaner et al., 2005, 2006). Confirming the Th2 paradigm, neutralization by monoclonal antibodies *in vivo* implied that CD8+ and γδ+ cells and IFN-γ (all Th1-response related) retarded the potential development of naturally acquired immunity against *Tc*L3 (McClure et al., 1995).

More effective resistance to helminth infections in ruminants is also associated with increasing levels of serum and mucosal, parasite-specific IgG1, IgA and IgE (Smith et al., 1983; Bendixsen et al., 2004; Pernthaner et al., 2005; 2006; Shaw et al., 2009; Hein et al., 2010). The effector component of immunity is consistent with an "allergic" inflammatory response involving mucosal mast cells and eosinophils (Stewart, 1955; Emery, 1996; Emery et al., 1993; Hein et al., 2010) that secrete inflammatory mediators which ultimately paralyse worm motility preventing L3 establishment (Jones et al., 1994), suppress egg production, remove adult worms (McClure et al., 1992) and perhaps disorientate incoming L3. Immunity in sheep to gut worms appears to be associated with increased enteric nerve fibre number and metabolic activity (Stewart et al. 1995b), and with hypertrophy and hypercontractility of local smooth muscle upon worm antigen contact (McClure, unpublished; Tremain and Emery, 1994) to enhance peristalsis and invoke mucus production, such that the intestinal wall thickens in GIN-immune sheep (see McClure, 2000). Mucosal mast cells (MMC) isolated from the small intestine of immune sheep released protease, leucotrienes and histamine after incubation with *Tc*L3 extracts (Jones et al, 1992; Bendixsen et al., 1995). Pre-formed (granular) mediators are released within 1h while those synthesised *de novo* are released in 24-48h (Fig. 3; Bendixsen et al., 1995). Abomasal MMC have not been isolated in sufficient numbers to examine responses to *Tel c*L3 and *Hc*L3. The kinetics and magnitude of protease release was consistent with a principle role of MMC mediators in rejection of *Tc* (Bendixsen et al., 1995), while substance P and leukotriene C4 inhibited migration of *Tc*L3 *in vitro* at physiological concentrations detected in sequential contents from fistulated ovine small intestines during development of immunity to trickle infections (Jones & Emery, unpublished data). The levels of MMC sensitisation in the small intestine are highest around the site of establishment of *Tc* (first 5m), diminishing distally as judged by the quantities of sheep mast cell protease (SMCP) released *in vitro* (Bendixsen et al., 1995). This effect is consistent with the ability of paralysed worms to recover and establish further down the GI tract (Harrison et al., 1999). Despite these strong associations with immunity and worm rejection, unequivocal evidence of a protective immune response has only been demonstrated for antibody to the carbohydrate larval antigen (CarLA) found on many nematode L3s (including *Hc*L3). The CARLA response, essentially a "neutralising antibody" preventing *Tc*L3 establishment, is a

selective option to avoid "immune-mediated scouring" which is reported as a sequel to *Tc*L3 immunity in some Australian and NZ flocks (Williams, 2010). This appears associated with eosinophilia in the intestinal mucosa (Larsen et al., 1995) and a conventional MMC-mediated rejection response.

Extrinsic inflammation and immunity to Tc

Several unrelated observations have suggested that akin to the removal of Tc by non-specific "allergic" effector mechanisms associated with the release of inflammatory mediators, the induction of inflammation in the small intestine may compromise TcL3 establishment. These have included:

- Ground navy beans, given as 10% of pelleted diets and constituting 50% of digestible dietary protein, significantly (P<0.05) reduced parasitism in sheep given *T. colubriformis* L3 or harbouring a mature infection (D. Emery unpublished). Sheep were fed the diet for up to 22 days from the day of infection (L3) or from 3 weeks into a primary infection, and in both situations, parasitism was reduced by more than 60%. It is hypothesized that lectins within the navy beans increased mucosal inflammation in the same way that soya bean diets can induce diarrhoea in feedlot cattle.

- Oral delivery of attenuated *Salmonella typhimurium* aro A also provided variable levels of resistance, ranging from 35-50% against challenge with *Tc* (H. Brahmblatt, unpublished; Table 2).

- Similar levels of resistance were achieved by IP inoculation of liposomes (D. Emery unpublished) and supplementation of sheep diets with molybdenum also decreased levels of parasitism following challenge (McClure et al., 1999).

- Feeding of different types of lipids also influences the rate of acquisition of immunity. Lambs fed n-3 fatty acids during a primary infection with *T. colubriformis* had significantly (P<0.05) more worms after challenge (13048±1868) than those fed n-6 (6995±2033) or neutral (5307±1906) lipids. As development of protective immunity to *T. colubriformis* is thought to involve local inflammation, this result is consistent with the hypothesis that dietary omega-3 lipid is exerting an anti-inflammatory effect on the local immune response (McClure, 2000).

Immunity to *Teladorsagia* (*Telc*)

As observed with trickle infections with *Tc*L3 and *Hc*L3, immunity in lambs dosed daily with 1000 *Telc* L3, slowly develops from 4 weeks against incoming L3 (Seaton et al., 1989). There are two major manifestations of resistance to *Tel c*: the regulation of worm growth and the control of worm establishment, reproduction and survival. The regulation of worm size and fecundity is strongly and consistently associated with IgA activity especially against fourth-stage larvae (Stear et al., 1995; Strain et al., 2002; Martinez et al., 2002). This effect is so strong and consistent that it is likely causal because there is no other immunological mechanism that is so strongly associated with IgA activity. Eosinophil number is also associated with worm length and fecundity (Stear et al., 2002) and it is likely that eosinophils and IgA interact to influence worm growth and fecundity. Eosinophils have been implicated in resistance to infection to Hc (Gonzales et al., 2011; Meeusen & Balic 2000). Eosinophil activity against *Hc*L3 is not surprising, as inoculation of soluble extracts of *Hc*L3/*Tc*L3 into the mammary gland of immune and non-immune ewes elicits within 24h, an exudate comprising

>90% eosinophils, confirming the chemo-attractive activity of *Hc*L3 products (Adams & Colditz, 1991). Eosinophils play a much weaker role in resistance of mice to parasitic infections (Urban et al., 1991) but this may reflect the fact that mice eosinophils lack the IgA receptor (van Egmond et al., 2001).

The other major mechanism of resistance to *Tel c* is the IgE and MMC-mediated control of worm establishment and survival (Stear et al., 1995), which is elicited in association with activation of pro-inflammatory Th17 cells in the mucosa (Gossner et al., 2012). Again, this association is so strong and consistent that it is likely to be causal rather than acting as a marker for some other response. IgE activity against third-stage larvae seems to be most important (Sinski et al., 1995). The number of molecules recognised by IgE seems to be relatively small; two-dimensional Western blotting identified only 9 molecules although there is evidence for at least two other allergens (Murphy et al., 2010). For comparison, 155 molecules were recognised by IgA on third-stage larvae (Ellis et al., 2014).

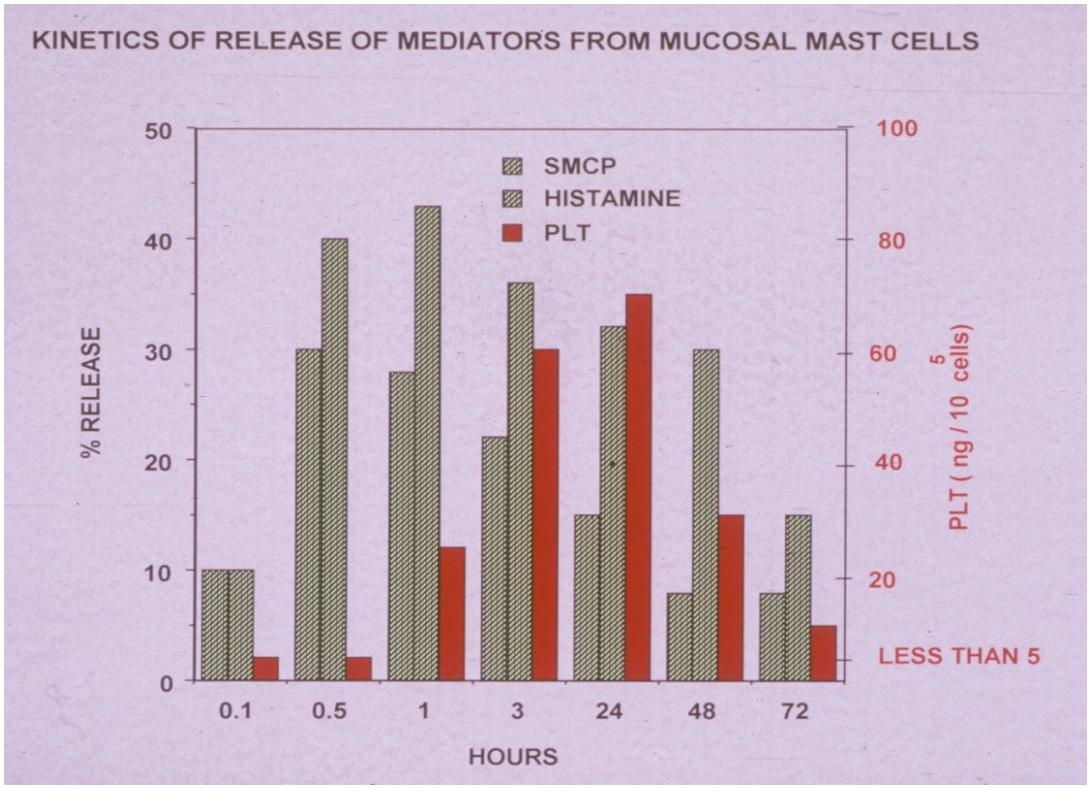

Fig. 3. Kinetics of release of pre-formed sheep mast cell protease (SMCP), histamine and de-novo synthesized peptidyl leukotrienes (PLT) from mucosal mast cells (MMC) isolated from the small intestine of immune sheep and incubated with soluble proteins for TcL3.

2.2.2 Epidemiological influences on GIN immunity

Mucosal immune mechanisms are influenced by intrinsic and extrinsic factors including physiological factors such as age, liveweight, gender and pregnancy/ lactation, and external factors such as nutrition and weaning stress. Nutritional requirements for the development of optimal mucosal immunity as well as growth and production are also critical (McClure, 2000; Coop & Kyriazakis, 2001).

Naturally acquired immunity to scour worms is transient and requires relatively constant ingestion of GIN to maintain optimal levels of efficacy and efficiency, as exemplified by variability in anti-CarLA IgA titres (Shaw et al., 2012) and the slow acquisition and expression of age-related resistance (Adams, 1993). Consequently, seasonal fluctuations in the levels of pasture/ ingested L3, as well as suppressive drenching regimes restrict the development of immunity in young lambs (Barger, 1988; McRae et al., 2015).

Liveweight (nutrition/ birth weight) also presents an issue for GIN immunity. A weaning weight of 23 kg has been deemed the critical minimum for optimal survival rates and production of Merino weaners (Holmes & Sackett, 1990). Moreover, a negative correlation between initial liveweight and *Tc* worm count after challenge was observed in 8-month-old Merino lambs, while those lambs with an initial liveweight less than 22 kg had higher FWEC and worm counts during infections than their heavier cohorts (McClure et al., 1999).

PPRI: An important component of the epidemiology of *Tel c* infections is the periparturient relaxation in immunity (PPRI) to the parasite observed in ewes around lambing (Gibson, 1973). Using FINEC data obtained over a 4-year period from a Scottish Blackface flock on an upland farm in the UK, Singleton et al., (2011) developed a model of Tel c infection which incorporated the effects of immunity on parasite establishment and fecundity. The model, which accurately reproduced observed measurements, demonstrated that larval contamination of the pasture was primarily derived from the large numbers of eggs excreted by ewes during the PPRI period (Singleton et al., 2011). In the UK, the PPRI is thought to impact parasite transmission by the resumed development of immunologically-inhibited fourth stage larvae in previously infected ewes, augmented by further ingestion/ development of new infections derived from overwintered L3 on pasture (Gibson, 1973). Suppression of this periparturient source of infection is currently addressed in practice by the administration of anthelmintic to ewes around lambing (Taylor et al., 1993; Sargison et al., 2012); however, this is likely to increase selection for anthelmintic resistance as the lambing treatment is applied when refugia levels of the nematodes on pasture are relatively low (Sargison et al., 2012). There are further concerns regarding the presence of anthelmintics with persistent activity in milk, which will achieve sub-optimal concentrations in lambs and hence select for resistance in parasites that they subsequently ingest (Leathwick et al., 2015). Nematode egg excretion around lambing has also been shown to be reduced in ewes by increasing the quantity and quality of dietary protein offered in the diet. Mechanistically, this is thought to work through balancing metabolisable protein resources for immune function and gestation/lactation (Donaldson et al., 2001; Houdijk et al., 2001, 2003; Sakkas et al., 2012). However, the impact of nutritional supplementation on the PPRI in nematode infections varies amongst different breeds of sheep (Kidane et al., 2010). Reducing energy demands in the periparturient period (for example, in ewes with a single rather than twin lambs) can also reduce the effect of the PPRI on nematode egg shedding (Houdijk, 2008; Houdijk et al., 2006). Both increasing the levels of nutrition and reducing energy demands by limiting lamb numbers imposes a substantial economic cost on sheep farmers, with vaccination potentially offering a more economical alternative.

2.2.3 Genetic influences on GIN immunity

Research into inherent variation among livestock in resistance to parasites has been carried out since at least since the beginning of the 20th Century (Francis, 1966). The heritability is one of the

most important measures of genetic variation. In the narrow sense used by animal breeders, this measures the proportion of the total variation which is inherited. Interactions between alleles at the same locus or among alleles at different loci are not inherited. Inherited genetic effects reflect the average effect of each gene in the population and are also known as additive effects while interactions among alleles are known as non-additive effects.

The heritability of resistance to gastrointestinal nematodes has been estimated in Australian sheep deliberately challenged with *H. contortus (*Albers et al., 1987), *T. colubriformis* (Windon, 1996) and *T. circumcincta* (Yong et al., 1991). Studies in New Zealand (Morris et al., 1997), Spain (Gutierruz et al., 2010) Poland (Bouix et al., 1998) Kenya (Baker et al., 1999) and Scotland (Bishop et al., 1996) have used natural infections; while studies in France and Kenya have used a combination of natural and deliberate infection (Assenza et al., 2014). Heritabilities for resistance to gastrointestinal nematode infection have also been estimated in cattle from Australia (Stear et al., 1988; Mackinnon et al., 1991) and the USA (Leighton et al., 1989).

The research on heritability and subsequent research on genetic markers has produced a number of general conclusions. Resistance to gastrointestinal nematode infection is a genetically complex trait because it is influenced by many genes and multiple non-genetic effects such as nutrition and prior exposure. The heritability is similar for responses to natural or deliberate infection. Deliberate infections require more effort but are justified if the exposure to natural infection is unpredictable for example because of weather. The heritability of a single faecal nematode egg count generally ranges between 0.1 and 0.4 which is sufficient to justify a selective breeding programme. If multiple egg counts are made, this improves the heritability. Faecal egg counts are the most commonly used way to assess resistance but other markers exist which may be cheaper and more heritable such as packed cell volume (Albers et al., 1987), parasite-specific plasma IgA activity (Strain et al., 2002) and salivary IgA activity against CarLA (Shaw et al., 2013; Shaw et al., 2012).

There are three strategies for exploiting genetic variation: breed substitution, cross-breeding and selection within populations although these strategies are not mutually exclusive. There have been several comparisons of different breeds of sheep although most of these have involved indigenous breeds of sheep. For *H. contortus,* a number of relatively resistant breeds have been identified including St Croix (Gamble et al., 1992), Gulf Coast Native (Bahirathan et al., 2000), Red Maasai (Mugambi et al., 1996) and Barbados Blackbelly (Yazwinski et al., 1981) while Hampshire Down sheep appear relatively susceptible (Wallace et al., 1995). For *T. circumcincta*, Texel sheep are more resistant than Suffolk sheep (Good et al., 2006).

There has been very little work on quantifying the benefits of cross-breeding for resistance to nematode infection. In contrast, there have been several studies that have conclusively demonstrated the feasibility of selective breeding for resistance to gastrointestinal nematodes (Bisset et al, 2001; Woolaston & Piper, 2010; Woolaston & Windon, 2001; Karlsson & Greef, 2006). Selective breeding for enhanced resistance to nematodes is now being applied in commercial flocks in Australia, New Zealand and the UK. The major concerns are balancing the relationship with productivity and managing the transition to genomic selection. Mrode et al. (2018) recommend a cost-benefit analysis for farmers in the third world to decide the most appropriate method of selection but the arguments also apply to sheep breeders with extensively managed flocks.

2.2.4 Untoward effects of GIN immunity

***Tel c* and immune-mediated pathology**. The inflammatory effector arm of the immune response appears to be responsible for much of the pathology following *Tel c* infection (Stear et al., 2003). Immune suppression abolishes many of the clinical signs (Greer et al., 2005). Infection causes a relative protein deficiency that leads to reduced weight gain or even weight loss. This is due in part to a reduction in appetite (Stear et al., 2000; Wallace et al, 1998). There is also a loss in digestive efficiency. Lesions in the epithelial barrier allow a loss of protein and in addition protein is diverted to tissue repair and immune and inflammatory processes. Protein supplementation of the diet can prevent the appearance of clinical signs (Stear et al., 2000; Coop & Kyriazakis, 1999) which argues strongly that pathogenesis is a consequence of the relative protein deficiency. Prolonged and sustained infection subverts the repair process into a pathogenic mechanism. This would be offset by a vaccine that reduced infection levels or targeted larval stages to subvert establishment.

***Telc* and *Tc* " immune scours"**. The larval hypersensitivity syndrome (as discussed above) occurs where the ingestion of nematode L3 in immune animals aged >12 months, incites an inflammatory response which prevents the development of the larvae to adult worms, but results in scouring in a proportion of sheep (Larsen et al 1999; Jacobsen et al 2009; Williams & Palmer, 2012). The only recognisable differential pathogy is the accumulation of eosinophils in the submucosa of affected individuals (Larsen et al., 1999). Attempts to block and abrogate the eosinophil reaction with anti IL-5 MAbs were unsuccessful (Emery & Larsen unpublished). A "de-sensitisation" strategy involving 37 oral administrations of 1000 sonicated *Trichostrongylus* spp L3 over 13 weeks prior to challenge with 7000 L3, abrogated mucosal eosinophilia but increased worm counts by 42% (O'Handley, 2013). No scouring was observed in the penned sheep. Both *Telc* L3 and *Tc*L3s can induce the reaction, which is not observed in NZ sheep with high anti-CarLA immunity (R.Shaw, pers. comm). This may suggest that immunity associated with CarLA may operate differently from the presumptive cause of the scouring mechanism emanating from the mast cell-IgE response. The response is similar to the historical "self-cure" reaction to *Hc*, whereby incoming L3 incite removal of established adult worms (Stewart, 1955).

3 Adjuvants and Immunostimulants for ruminants

The variety of adjuvants (including "immunostimulants" and "immuno-enhancers") available to veterinary vaccinologists far exceeds the limited array for human vaccines. This arises from the various degrees of tissue irritability from many preparations. For the rational formulation of vaccines it is essential to understand the chemistry, properties and activity of the various adjuvants (especially emulsions) required to induce the desired protective response(s) where these are also known. For ruminants, this broad field is slowly moving from empiricism to precision, in tandem with understandings of mucosal and systemic immunity.

3.1 Conventional adjuvants

Broadly, conventional veterinary vaccines are oil-based emulsions (e.g. incomplete Freund's adjuvant- IFA) or other compounds, or combinations of these 2 major classes. The "type" of oil influences the extent of tissue reactions, with muramyl-dipeptide (MDP) in complete Freunds adjuvant (FCA) causing large lesions which are not correlated with antibody titres (Stewart et al., 1985). More recent versions in the Montanide series are less irritant with more consistent

immunostimulation. Oil-based emulsions provide a local “inflammatory” tissue depot, with slow release of antigen over months, as indicated when removal of the injection site and regional lymph node up to 3 months later caused significant reductions in, and persistence of antibody titres (Lascelles et al., 1989). Indeed, it was possible to achieve a heightened, antigen-specific, IgG response by inoculating an unrelated soluble antigen into an oil-based granuloma for up to 3 weeks after the initial SC injection (Emery, unpublished), taking advantage of the “sensitization” and innate inflammatory stimulus from the lesion. The depot, slow-release effect was consistent with a delayed but prolonged IFN-γ response from the draining lymph node, when compared to shorter-acting adjuvants such as QuilA (saponin, QS21), dextran sulphate (DXS) and dodecyl-ammonium bromide (DDA) (Emery et al., 1990).

A range of other compounds and chemicals have been evaluated in sheep as part of empirical approaches to achieve successful outcomes, often in probing to identify protective responses from the vaccine formulation in models such as footrot (Stewart et al, 1989, where agglutinating titres are known to be the protective response), liver fluke (*Fasciola hepatica*; Spithill & Dalton, 1998) and worm vaccines (Tables 3-5). Most popular and well-characterised are the saponins (QuilA, QS21), alhydrogel (AH) and DXS. Quil A and DXS given SC are acutely inflammatory, giving rise to short sharp rises in antibody titres and IFN-γ responses (Emery et al, 1990). Given that alhydrogel (AH) did not elicit IFN-γ production after inoculation or from primed lymphocytes (Emery et al., 1990), this adjuvant was presumed to elicit a Th2-type response, sought as a protective response by worm vaccines (Emery, 1996). To stimulate ovine mucosal immunity, intraperitoneal (IP) inoculation of IFA was shown to stimulate intestinal immune responses (Beh & Lascelles, 1981), inducing widespread, ectopic, lymphoid aggregates in the mesentery.

The empirical nature of adjuvant use in ruminant nematode vaccine development to date is reflected in the lack of data directly comparing the effect of different adjuvants on subsequent vaccine performance, coupled with lack of definition of the protective response needed to be generated. Where this has been done, the results are often inconsistent. For example, using a native extract of native *H. contortus, Hc*sL3, it was shown that formulation with Aluminium Phosphate induced significant levels of protection against *H. contortus* challenge, but the effect was abrogated when Quil A was added to the vaccine formulation (Piedrafita et al 2013). In a similar trial using a recombinant version of Hc23, protection of 70% was similar in groups vaccinated with the antigen in either Aluminium Hydroxide (AH) or Quil A, although in this trial, cumulative FEC was reduced by around 45% in lambs vaccinated with Quil A alone (González-Sánchez et al 2018). In contrast, vaccination of cattle against the abomasal parasite *Ostertagia ostertagi*, which is closely related to *T. circumcincta*, with either native Aspartyl-proteinase (ASP) or ES-thiol, a cysteine protease rich fraction of adult *O. ostertagi* excretory-secretory products, induces protection if formulated with Quil A but not with AH (Geldhof et al 2004; Gonzales-Hernandez et al 2016). Similar outcomes occurred with the induction of protective immunity following the immunisation of cattle with surface antigens from *Trypanosoma brucei* (Wells et al., 1982). Thus it appears that Th2 promoting adjuvants (e.g. those based on aluminium salts) are not always associated with optimal protection, and that empirical testing of adjuvants where the adjuvants are directly compared, is still required.

3.2 Alternative delivery strategies and formulations for mucosal immunity

Given that GIN reside at mucosal surfaces and that immunity to these parasites is strongly associated with immune effectors at the mucosal site, significant, and to date largely unsuccessful, efforts have been made to develop vaccine adjuvants and/or delivery systems which are capable of inducing mucosal immune responses local to the site of infection.

The most effective way of inducing mucosal immune responses is by administration of vaccines onto the mucosal surface where antigen is primarily sampled by specialised cells (microfold or M-cells or goblet cells) which deliver antigen to underlying dendritic cells (DCs). In the case of M-cells, these localise to the follicle-associated epithelium (FAE) overlying organised lymphoid tissue within the mucosa itself (mucosa associated lymphoid tissue; MALT). DCs subsequently present antigen to T cells within MALT or lymph nodes draining the mucosal site to initiate the adaptive immune response (Neutra et al. 2001). During this process, expression of tissue-specific adhesion molecules and chemokine receptors are induced on activated T and B cells (Table 2) which allow lymphocytes to home back to mucosal sites through recognition of counter-receptors expressed on mucosal endothelial cells and chemokine-mediated tissue migration to the mucosal site. In contrast, vaccines delivered via sub-cutaneous or intra-muscular routes do not generally confer mucosal homing on lymphocytes and therefore are poor at inducing mucosal immune responses (McClure, 2009).

Table 2. Summary of homing receptors, adhesins and chemokines involved in lymphocyte trafficking to intestinal and non-intestinal sites (adapted from Kunkel et al., 2003).

Homing site	Lymphocyte homing receptors	Endothelial receptor	Chemokine(s)
Small intestine	α4β7 integrin, CCR9	MADCAM-1	CCL25
Large intestine	α4β7 integrin, CCR10	MADCAM-1	CCL28
Non-intestinal sites	α4β1 integrin, CXCR3, CCR4, CXCR4, CCR10	VCAM-1	CXCL9/10, CXCL12, CCL27, CCL17, CCL28

One of the key problems facing mucosal vaccination is that mucosally-applied antigens face a formidable array of host defences: they may be diluted in mucosal secretions or trapped in mucus, excluded by epithelial barriers or agglutinating antibody, and in the case of orally administered vaccines, face large dilutions within the rumen and enzymatic and proteolytic degradation in the abomasum (McNeilly et al., 2008). Furthermore, uptake of antigens in the intestine often results in immune tolerance (Mayer & Shao 2004). Mucosal vaccines must therefore both deliver adequate levels of antigen across the mucosal barrier as well as activate appropriate signalling events to induce adaptive immunity and not immune tolerance.

3.2.1 Mucosal delivery systems

Route of delivery: Mucosally-primed lymphocytes not only home back to the mucosal site of immune induction but also to distant mucosal sites, leading to the concept of the “common mucosal immune system” (McClure 2000). Given the challenges associated with oral vaccine delivery in ruminants, the common mucosal immune system may be exploited to induce mucosal immune

responses local to the GIN by delivering antigens directly to accessible mucosal sites such as the terminal rectum or nasal cavity, which are both rich in MALT. A series of trials have been conducted in which *Tc* antigens, either L3 homogenate or a 17kDa recombinant protein, were delivered in either cellulose or chitosan gels intra-rectally in sheep and conferred some degree of protection against subsequent TcL3 challenge (McClure, 2008). In some of these trials, rectal immunisation induced parasite-specific antibodies in the jejunum which correlated with parasite faecal egg count and worm burden, providing evidence that protection was related to induction of a common mucosal immune response.

It has been previously shown that intra-nasal vaccination of sheep with PP2Ar (a recombinant part of the catalytic region of serine/threonine phosphatase 2A from the hookworm *Angiostrongylus costaricensis*) together with *E. coli* bacterial cell walls, induced protection against *Hc* and *Telc* (>60% reduction in worm burdens). Furthermore, intra-nasal vaccination of pigs and mice with recombinant *Ascaris suum* antigens, As16 and As14, has been shown to reduce numbers of larvae recovered from the lung (Tsuji et al., 2001; 2003; 2004). These studies demonstrate the potential of intra-nasal vaccination to control GIN, although with *A. suum* vaccinations it is unclear whether protection in these studies acted at the level of the intestine or through targeting larval migration within lungs.

Live vectors: The efficacy of live vectors in mucosal vaccines is largely due to their ability to survive the luminal environment, actively invade mucosal tissues and engage appropriate innate immune responses (including chemokines involved in lymphocyte homing, see Table 2) in order to direct subsequent adaptive immune responses to the mucosa. With regard to nematodes, most work on live mucosal vaccines has been conducted using attenuated strains of *Salmonella typhimurium* in mice. In these studies, oral or intra-nasal delivery of attenuated *S.typhimurium* expressing a number of *Trichinella spiralis* antigens elicited robust Th1/Th2 and mucosal IgA and conferred protection against subsequent *T. spiralis* infection (Poma-Mera 2011; Wang 2016; Yang 2010; Lui 2015). However, there are some concerns over their safety and stability, and their efficacy in the face of pre-existing immunity to the vector.

Particulate delivery systems: The rationale behind particulate delivery systems is that formulation of antigens into particles both protects the antigen from degradation and increases uptake of antigen by MALT. Enhanced uptake of microparticles is largely due to uptake by M-cells which efficiently take up particles of <1µm in diameter. Little research has been conducted on the use of microparticles in nematode vaccines to date. However, in mice oral delivery of *T. spiralis* larval extracts or excretory-secretory products incorporated into methacrilic acid copolymers successfully induced mucosal IgA responses and protection against parasite challenge, indicating the potential utility of the approach (Dea-Ayuela et al., 2006). A number of microparticles have been tested in sheep, but not specifically for nematode vaccines. These include poly(D,L-lactide-co-glycolide) microparticles which were used to deliver *Toxoplasma* antigens via the nasal route (Stanley et al., 2004), and immunostimulating complexes (ISCOMs®), 40nm nanoparticles, which has been used to deliver viral antigens via oral or nasal routes (Coulter et al., 2003; van Pinxteren et al., 1999). The recent discovery of mucosal 'targeting' peptides which facilitate transcytosis of conjugated molecules across the sheep intestinal epithelium to dendritic cells in the lamina propria and Peyer's patches (Kenngott et al., 2016) raises the possibility that intestinal microparticle uptake could be enhanced by incorporation of these targeting peptides into particulate delivery systems.

Mucosal adjuvants: The induction of adaptive immune responses to inert mucosally-delivered antigens usually requires co-delivery of mucosal adjuvants. The most commonly employed mucosal adjuvants are Cholera Toxin (CT) and *Escherichia coli* heat-labile toxin (LT). Only CT has been tested in sheep and has been shown to induce IgA mucosal responses following delivery via rectal and intra-nasal routes (Jacobs et al 1999, Premier et al 2004; Stanley et al, 2004). Adjuvancy can also be an inherent property of the delivery system: for example chitosan gels used to deliver Tc antigens rectally possess adjuvant properties (McClure et al. 2008), and \(ISCOMs® have adjuvant properties as they are largely composed of the adjuvant Quil A (Morein et al., 2004). The use of Toll-like receptors ligands such as bacterial flagellins (Lee et al., 2006; Meeusen et al., 2013) and Cytosine-guanidine (CpG) DNA motifs (Gallichan et al., 2001) have also been successfully used as mucosal adjuvants, but not yet in sheep.

3.2.2 On the induction of Th2 responses in sheep

The standard mouse model of asthma involves multiple rounds of parental (subcutaneous-SC or IM) sensitization, usually with ovalbumin in alum adjuvant (a "Th2 adjuvant" for mice); followed by aerosol exposure to ovalbumin (Gershwin 2015). In sheep models of allergic asthma, SC immunizations with ovalbumin (Abraham 2008; Abraham et al., 1983), peanut allergen (Van Gramberg et al., 2012) and dust mite allergen (HDM), each given in alum adjuvant (Bischof et al., 2003) have each successfully induced antigen-specific IgE. In the dust-mite model, 50mg HDM resulted in approximately 60% of sheep developing high levels of HDM-specific serum IgE. Lower (5mg) or higher (500mg) doses of HDM were less effective. Experimental sensitized sheep segregated into high and low IgE responders to HDM, and those with high specific IgE serum titres (allergic sheep) generated an elevated and prolonged eosinophilia in bronchial alveolar lavage fluids when challenged locally with HDM, compared with non-allergic and control sheep (Bischof et al., 2003). This would suggest that if antigen-specific IgE responses provided protection against GIN, these protocols should induce Th2 responses in sheep mucosa.

Numerous studies have confirmed that nematode and many ectoparasite infections typically induce a T-helper 2 response with elevated IgE responses (see Shaw et al., 2009). Sheep suffering nasal bot (*Oestrus ovis*) infestation also develop local allergic responses and IgE to antigens produced by the developing larvae (Anguol-Valadez et al., 2011). Following *T. colubriformis* challenge, immune animals had higher levels of total IgE in intestinal lymph than non-immune sheep, low FWEC sheep had higher concentrations of total IgE than high FWEC sheep, and field-primed animals had higher total IgE levels than nematode naïve animals, arising from stage-specific induction of IgA and IgE responses in regional mesenteric lymph nodes (MLN) after infection (Pernthaner et al., 2006). Similar observations have been made following *Telc* challenge studies in which elevated IgE responses to L3 but not adult antigens were detected in abomasal lymph samples from immune vs. non-immune sheep (Huntley et al., 1998). Serum IgE obtained from high and low FWEC selection line lambs bound to Western blotted *Tc* L3 antigens of 40 (galectin), 31 (Aspin) and 21 kDa (Shaw et al., 2003; Bendixsen et al., 2004). Levels of specific IgE to *Tc* L3-ES was heritable (h^2 = 0.36) and showed a negative genetic correlation with FWEC (ca -0.31; Shaw et al., 1999). By comparison, IgA titres to CarLA were both moderately heritable (0.28 ± 0.10) and genetically correlated negatively with FWEC (-0.57 ± 0.20; Shaw et al., 2013).

As a consequence of the strong associations between Th2 responses and GIN immunity, methods to induce Th2 responses have been attempted by various routes. Sheep dosed intranasally with *E.coli* -expressed, serine/threonine protein phosphatase (PP2A) had >60% reduced *Hc* and *Telc* worm burdens after challenge, but *Tc* was unaffected (Fazwi et al., 2013). It was also noted that *E.coli* cell walls also reduced *Telc* burdens by around 45% (Fazwi et al., 2013). In another study using fractionated *Hc* adult ES (excretory-secretory) antigen and aluminium adjuvant, ES-specific IgE levels in serum increased after SC immunisation and after challenge infection (Bakker et al., 2004), but there was no correlation between protection (worm numbers) and antibody levels in serum or mucus. In a study of mucosal delivery of native and recombinant *Tc* proteins, McClure (2009) induced significantly elevated serum IgG, IgA and IgE after delivery of parasite antigens (without adjuvant) to the epithelium underlying rectal Peyer's patches, but once again, protection was around 50%, consistent with previous trials using the same antigen.

However, despite the correlations, the precise role of IgE responses in prevention, removal or stimulation of scratching (ectoparasites) for endo- and ectoparasites is still unresolved. What is clear is that:

1. antigens on parasites recognised by IgE or IgA from immune animals do not possess intrinsic "allergic" potential in that they do not readily generate IgA or IgE when presented in purified or recombinant- adjuvanted forms or are not protective;

2. moieties or formulations which induce Th2 responses in rodent models do not reliably extrapolate to ruminants; and,

3. vaccine formulations which induce mucosal Th2 responses in ruminants are not readily available and we don't know how to make them.

While the mechanisms by which Th1 immunity is induced are largely understood, until recently the mechanism underlying initiation of Th2 immunity has been unclear. Recent studies in murine nematode infection models now indicate a critical role for tuft cells, a specialised and relatively rare epithelial cell type, in the initiation of Th2 immunity to GI nematodes (Gerbe et al. 2016; Howitt et al., 2016; von Moltke et al., 2016). Tuft cells appear to be the primary source of the Th2 alarmin, IL-25, which activates innate lymphoid cells (ILCs); ILC2-derived IL-13 acts in a positive feedback loop to expand tuft cell numbers. Once sufficient IL-25 and IL-13 are generated, Th2 immunity is induced. Tuft cells have now been identified in sheep and expand during *Telc* infection in a similar manner to that observed in mice (Hildersley et al., unpublished). As tuft cells are chemosensory, it is possible that their activation to produce IL-25 raises the possibility that tuft cells are sensing 'helminth-associated molecular patterns', or other molecules which are indirectly induced in the gut lumen following parasite infection. If identified, these tuft cell activating molecules could be used as adjuvants to drive more effective Th2 immunity.

3.2.3 Immune modulators for redirection of systemically induced immune responses to mucosal surfaces

An as yet unexploited method of inducing mucosal immune responses is through the use of specific adjuvants within systemically delivered vaccines which target the immune response to mucosal sites. The systemic route of immunisation would have benefits over mucosal delivery including ease of administration and delivery of a known quantity of antigen into the body. One example of a

mucosal-targeting adjuvant is Vitamin D3 which has been shown in mice, pigs and cattle to induce mucosal antigen-specific IgA responses when incorporated into systemically delivered subunit vaccines (Daynes et al., 1996, Van der et al 2001, Asper et al., 2011). Other examples include toll-like (TLR) ligands, poly I:C (TLR3 ligand) LPS (TLR4 ligand), and Flagellin (TLR5 ligand), which have been shown to induce mucosa IgA responses following systemic delivery (McNeilly et al., 2008, Enioutina et al., 2008). In the case of TLR3 and TLR4, this was associated with upregulation of 1α-hydroxylase in dendritic cells which converts circulating 25(OH)D3 to calcitriol, the active form of vitamin D3. Thus exploiting vitamin D, either by direct inclusion into systemically delivered vaccines or incorporation of Pattern Recognition Receptor (PPR) ligands which upregulate vitamin D activity, may be a useful approach to enhance mucosal immune responses in nematode vaccines.

4 Vaccines

4.1 Introduction: antigen discovery and types of vaccines

There are 5 main categories of vaccines for the control of pathogens in humans and livestock:

1) Chemically (anthelmintic) abbreviated infections ("truncated" infections) .

2) Pathogens attenuated by irradiation or culture (or use of closely-related, less pathogenic organisms).

3) Protein subunit/nucleic acid subunit vaccines – pathogen molecules which can be either native proteins, protein complexes or recombinant subunits.

4) Pathogens killed by chemicals or by heat.

5) Toxoids - inactivated compounds where these (rather than the organisms) cause disease.

The development of prototype and commercialised vaccines (successful against *Hc* in the case of Barbervax®) against scour worms has focussed on 1- 3 of these categories and these are further explored below:

4.2 Life-cycle targets for worm vaccines

A broad examination of the expression of naturally-acquired immunity against the principal GIN *(Hc, Tc* and *Tel.c*) indicates that the most vulnerable stage and against which developing immunity is first expressed are incoming L3, drastically reducing establishment rates (Barger et al., 1985; Dobson et al., 1990) or larval development (Seaton et al., 1989). The results also indicate a common inductive time-frame and effector mechanism (Stewart, 1955; Emery et al., 1993; Harrison et al., 1999). The infective/parasitic L3 stage of GIN parasites would be the most obvious to target for scour-worm vaccines, perhaps offering the greatest opportunity for cross-protective (or multi-valent) vaccines, but also removing the parasite to negate the pathophysiology associated with worm development (Barker et al., 1982; McKellar et al., 1993) and the collateral damage of immune responses against established worms (Stear et al., 2003; Greer et al., 2005; MLA, 2017). However, since low doses of incoming worms can often escape the inductive threshold for the most established immunity (Smith et al., 1983; Dobson et al., 1990), information on protective immunogens from later stages of GIN are also needed.

4.3 Immunity induced by types of vaccines

GIN immunity is rarely "sterile". Under natural conditions with low dose challenge, some worms establish and may be retarded, even in highly immune or immunised animals (Smith et al, 1983). A quantum of 3000-4500 infective GIN L3 is considered a threshold to ignite the immune response (Dobson et al., 1990). Comparatively, GIN with developmental stages in tissues (eg *Tel c, O.col*) are more vulnerable to systemic attack (suppression/ retarded development) than those residing on the mucosa (*Tc, Tv, T.axei, C.ovina*).

4.3.1 Chemically abbreviated (truncated) infections

Chemically abbreviated (truncated) infections have been used to investigate the "gold standard" for protective immunity as well as examine the ability of larval and adult antigens to establish comprehensive protection and cross-protection. The outcome of repeated rounds of *Tc*L3 infestation (3 single infections of 30,000 TcL3) generated solid resistance to homologous *Tc*L3 and adult worm challenge in hoggets, rejection occurring within 3-5 days (Emery et al., 1992a). Sheep given similar 4 x 7d and 4 x 10d larval infections, rejected *Tc*L3 challenge but rejected only 50% of adult worms while sheep given 4 x 4d infections remained fully susceptible. This indicated that stage-specific antigens produced by early L3 and L4 stages of *Tc* effectively immunized sheep against a larval challenge, while sheep immunised with adoptively transferred adult worms took 7-10 days to reject the *Tc*L3 challenge, presumably acting as "adult-stage" antigens were produced (Emery et al., 1992b). Such approaches proved less successful in 1month old lambs (McClure et al., 1998). However, similar truncated infections also induced >90% protection against *Tc*L3 ingested from pasture (Stankiewicz et al., 1996; Harrison et al., 2003), with "immunity-induced" weight loss associated with increased levels of gut eosinophils (Stankiewicz et al., 1996). By comparison, this approach was unsuccessful against homologous *Telc*L3 challenge (Harrison et al., 2003) but successful against HcL3 (Robinson et al., 2010.).

Most interestingly, McClure (2009) administered 18 doses of 1200 TcL3 (in cellulose gel; 3 per week for 6 weeks) to the rectal lymphoid tissue and mucosa, generating >88% protection against a challenge infection of 30,000 *TcL3*, and demonstrating that "reverse vaccinology" through the caudal end was feasible! Most importantly, this approach also demonstrated the operation of the mucosal immune system, opening the possibility for routes of vaccination other than oral or IP (see section 4.2).

4.3.2 Radiation-attenuated vaccines

Following the successful immunisation of calves against bovine lungworm (*Dictyocaulus viviparus*) using only 2 doses of 1000 irradiated L3 (Jarrett et al, 1959; later Dictol®), Dineen et al (1978) utilised irradiated *TcL3* dosing protocols to select Merino weaner lambs into resistant ("high responder") and susceptible ("low responder") genotypes. Successive generations of the model were utilised to elucidate the mechanisms of GIN resistance. While this approach successfully segregated response phenotypes, the 2 x 20,000 TcL3 vaccination protocol was unrealistic for the field. Over a similar timeframe, it was established that immunisation of mature sheep (>7 months old) with irradiated *HcL3* or *TcL3* conferred high levels of protection against challenge with the homologous species (Jarrett et al., 1961; Dineen et al., 1977; Smith & Christie, 1978). Progress and doses were reviewed by Bain (1999).

In trials with *Telc*, mature (8 month old) Greyface-Suffolk cross sheep were immunised with two doses of 10,000 gamma-irradiated infective larvae (L3) four weeks apart and then challenged, four weeks later, with a bolus of 10,000 non-irradiated larvae (Smith et al., 1982). At post mortem, four weeks after the challenge dose, the adult worm burdens of immunised sheep were 60% lower ($P<0.05$) than those of sheep which had not received the immunising doses and peak faecal egg output was reduced by 65% in immunised sheep (Smith et al., 1982). When irradiated worms were removed by anthelmintics one week prior to challenge, the protective effect was lost, demonstrating the need for concomitant infection with the irradiated larvae to allow protection (Smith et al., 1982).

Similar levels of protection have also been demonstrated by immunising 3-5 month old lambs with two oral doses of 50,000 *Tel.c L3* which had been attenuated by UV irradiation (Bezubik et al. 1980, cited in Wedrychowicz et al., 1992).

4.3.3 Native Antigen-based vaccines

4.3.3.1 Parasite extracts

Barbervax®

The gut membrane-associated glycoprotein complexes H-gal-GP and H11 have consistently conferred protection in experimental trials and preparations of *Haemonchus* integral gut membrane proteins containing both H11 and H-gal-GP, when used as vaccines in sheep in controlled field trials, reduced anaemia, prevented deaths and reduced the contamination of pasture with infective larvae (LeJambre et al., 2008; Table 3). Preparations containing these antigens in microgram quantities conferred high levels of protection, allowing the commercial exploitation of a native vaccine (reviewed in Nisbet et al., 2016a). The protective efficacy was less in goats (MLA project P.PSH.0672). The antigens present in Barbervax® are hidden antigens which would not normally be exposed to the host's immune response during infection so repeated vaccination is required to stimulate high antigen-specific circulating antibody levels. However, in areas where haemonchosis is currently controlled by using repeated anthelmintic drenches throughout the grazing season (e.g the Northern Tablelands), the required frequency of application of Barbervax® is no higher than that required for drenching. An advantage of the use of the hidden antigens which make up Barbervax® is that they can produce protective effects in situations where natural immunity is either weak or ineffective, such as in young lambs or in periparturient ewes (Le Jambre et al., 2008).

Table 3. Summary of vaccine trials for *Haemonchus contortus* using recombinant proteins (from Nisbet et al., 2016).

Antigen	Expression system	Adjuvant	Route of administration	% reduction FWEC*	% reduced worm Burden*	Challenge	Reference
H11							
H11-1	Baculovirus: *Spodoptera frugiperda* Sf21	None (Sf21 cell extract)	IM	Unknown	30[a]	Bolus 15,000 L3	Reszka et al., 2007
Combined H11-1 H11-4	*Caenorhabditis elegans*	QuilA	SC	No**	No**	Bolus 5000 L3	Roberts et al., 2013
Combined H11-4 H11-5	*C. elegans*	QuilA	SC	No	No	Bolus 5000 L3	Roberts et al., 2013
H-gal-GP							

HcMEP-1	*Escherichia coli* (GST-fusion)	QuilA	IM	No	No	Bolus 5000 L3	Smith et al., 2003a
HcMEP-3	*E.coli* (GST-fusion)	QuilA	IM	No	No	Bolus 5000 L3	Smith et al., 2003a
HcPEP1	*E. coli*	IFA	IM	No	No	Bolus 5000 L3	Smith et al., 2003b
HcPEP1	*E. coli*	QuilA	IM	No	No	Bolus 5000 L3	Smith et al., 2003b
Combined HcMEP-1, HcMEP-3, HcMEP-4 HcPEP1	MEPs expressed in *S. frugiperda* Sf9 insect cells; PEP1 in *E. coli*	QuilA	IM	No	No	Bolus 5000 L3	Cachat et al., 2010
CYS-1	*E. coli*	QuilA	IM	No	No	Bolus 5000 L3	Newlands et al., 2001
Others							
PP2Ar†	*E. coli*	*E. coli* Rosetta 2(DE3)pLysS insoluble fraction	Intra-nasal	unknown††	No	Bolus 10,000 L3 mixed species‡	Fawzi et al., 2013
Combined *Hco*-gal-m *Hco*-gal-f	*E. coli*	IFA	IM	37-48% [a]	41-46% [a]	Bolus 5000 L3	Yanming et al., 2007
Hc23	*E. coli*	AH	Unknown	83%[b]	85%[b]	Bolus 15000 L3	Fawzi et al., 2015
HcENO	*E.coli* (Thioredoxin-fusion)	IFA-Montanide ISA 61VG	IM+ SC	50%	50%	Bolus 5000 L3	Kalyanasud--aram et al., 2015
Combined Hc15/24	*E. coli*	DDA (± *Trichoplusia ni* extract)	SC	0-42%	0-65%	Bolus 5000 L3	Vervelde et al., 2002
Combined hmcp1, 4 and 6	*E.coli* (GST-fusion)	QuilA (+ *E. coli* BL21 extract)	IM	0-10%	24-38%	Bolus 5000 L3	Redmond and Knox, 2004; 2006
Hc-CPL-1	*C. elegans*	QuilA	IM	No	No	Bolus 5000 L3	Murray et al., 2007

All data are from published studies using sheep, unless otherwise stated.

* Reduction in FWEC or worm burden compared with adjuvant-only control; IM, intramuscular; SC, subcutaneous

**No = No significant difference (P<0.05) when compared with adjuvant-only control group

† PP2Ar is a 76 amino acid portion of the catalytic region of serine/threonine phosphatase 2A from *Angiostrongylus costaricensis*

†† Reduction in FWEC observed compared to adjuvant-only control at some sampling points post-challenge but species composition of nematode eggs unknown so effect cannot be attributed to vaccine efficacy against *Haemonchus contortus*.

‡ 4000 *Haemonchus contortus* L3; 4000 *Trichostrongylus colubriformis* L3 and 2000 *Teladorsagia circumcincta* L3

[a] Trial performed in goats

[b] No adjuvant-only control group, comparison is with un-immunised infected group

T.circumcincta

Table 4 below, provides a summary of vaccination trials using native and recombinant antigens to control *Telc*. Immunisation of 5 month old Finn-Dorset male lambs with a detergent extract of exsheathed L3 *Telc* (representing the surface antigens) formulated with Beryllium hydroxide adjuvant, followed by challenge with a bolus of 50,000 *Telc* L3, resulted in parasite burdens *post mortem* which were significantly reduced (by 72%, P < 0.01) when compared to challenged lambs which had not been immunised with either the antigen or adjuvant (Wedrychowicz et al., 1992; 1995). Somatic extracts of L3 in the same adjuvant gave only modest reductions in worm burden (30%) compared to the untreated control lambs (Wedrychowicz et al., 1992). In contrast, somatic extracts of L4/early L5 stage *Telc*, administered to 6 month old Dorset Horn lambs by repeated injection in Freund's adjuvant over a 3 week period, induced substantial levels of protection (66% reductions in adult worm burden, 44% reduction in FWEC) when challenged with a sustained trickle infection of 5,000 L3 daily over a 15 day period (Rose, 1978).

Immunisation of sheep with lectin-binding integral membrane glycoproteins derived from the gut of adult *H. contortus* results in very high, consistent levels of protection on homologous challenge (Smith, 1999; Newton & Munn, 1999) (see section on Barbervax®, below) but immunisation of 2 month old Suffolk-Greyface cross lambs with these Haemonchus antigens conferred no cross – protection against *Telc* challenge (Smith et al., 2001). When 9 month old sheep of the same breed were immunised with lectin-binding integral membrane glycoproteins derived from adult *Telc*, formulated with Quil A adjuvant, and challenged with a bolus dose of 5,000 L3 *Telc*, worm burdens and FWECs were reduced by only 8% and 28% respectively when compared to control sheep which had received adjuvant only (Smith et al., 2001). This finding, allied with the observation that the immunisation had induced high levels of circulating antibodies, led the authors to conclude that adult *Telc* did not consume sufficient quantities of antibody for the gut antigen approach to be effective (Smith et al., 2001). Based on the hypothesis that L4 *Telc* induce more localized inflammation at the site of infection than the adult worms, and therefore are exposed to higher quantities of ingestible immunoglobulin, Halliday and Smith (2011) immunised 4 month old Scottish

Mule lambs with lectin-binding integral membrane glycoproteins derived from L4 *Telc*, with Quil A adjuvant, and challenged the lambs with L3 *Telc* using either a bolus (5,000 L3) or trickle (500 L3 daily for 10 days) challenge. However, no significant reductions were observed in either FWEC or worm burden with either parasite challenge method (Halliday & Smith, 2011).

Table 4. Summary of vaccine trials for *Teladorsagia circumcincta* using native material and recombinant proteins.

Extract/ antigen	**Dose**	**Route of vaccination**	**Adjuvant**	**% reduction (eggs)**	**% reduction (worms)**	**Reference**
Mixed larval stage metabolites and ES from in vitro culture	300,000 larval equivalents	IM Orally (6 mth lambs) IM (9 mth lambs) IM (3 mth lambs)	IFA none IFA IFA none	81% 76% 0% 57% 47%	44% 46% 7% 60% 48%	Rose 1976 Rose 1978 Rose 1978
Live mixed stage larvae from in vitro culture	50,000 larvae	SC (6 mth lambs)	none	37%	44%	Rose 1976
Macerated worms L4/L5 from in vitro culture	30,000 larvae	IM in 6 month old lambs	IFA	44%	66%	Rose 1978
L3 soluble somatic proteins	~750µg	SC (5 month old lambs)	Beryllium hydroxide (BeOH)	- #	32%	Wedrychowicz et al., 1992
Detergent (CTAB)	~750µg	SC (5 month old lambs)	BeOH	- #	72%	Wedrychowicz et al., 1992

extract of xL3 surface						
Detergent (CTAB) extract of xL3 surface	~750µg	SC in 5 month old lambs	IFA	- #	31%	Wedrychowicz et al., 1992
L3 antigen precipitated by IgA from lambs immunised with CTAB extract of xL3 surface	~875µg	SC in 5 month old lambs	BeOH	- #	72%^	Wedrychowicz et al., 1995
L4 Con-A - binding fraction of a detergent-soluble membrane extract	34-48 µg	IM in 4 month old lambs	Quil A	29%	- #	Halliday et al., 2011
Oc-gal-GP (Peanut and ConA lectin binding fractions from Triton X-100 extracts)	140µg	IM	Quil A	28%	8%	Smith et al., 2001
8 recombinant antigen cocktail: Tci-APY-1 Tci-ASP-1 Tci-CF-1 Tci-ES20	50µg each protein	SC in lambs 3-7 months old at first vaccination	Quil A	70% 58% 0% 14% 49% 52% 0%	55% 56% 15% 3% 67% 64% 46%	Nisbet et al., 2013; Nisbet et al., 2019

Tci-MEP-1 Tci-MIF-1 Tci-SAA-1 Tci-TGH-2		SC in pregnant ewes	Quil A	47% 30% 44%	74% 50% ND*	Nisbet et al., 2016
4 recombinant antigen cocktail: Tci-APY-1 Tci-CF-1 Tci-ES20 Tci-MEP-1	50µg each protein	SC in lambs 4.5 months old at first vaccination	Quil A	0%	0%	Nisbet et al., Unpubl
4 recombinant antigen cocktail: Tci-ASP-1 Tci-MIF-1 Tci-SAA-1 Tci-TGH-2	50µg each protein	SC in lambs 4.5 months old at first vaccination	Quil A	0%	0%	Nisbet et al., Unpubl
2 recombinant antigen cocktail: Tci-APY-1 Tci-MEP-1	50µg each protein	SC in lambs 6 months old at first vaccination	Quil A	36% 3%	21% 34%	Nisbet et al., 2019
2 recombinant antigen cocktail:	50µg each protein	SC in lambs 6 months old at first vaccination	Quil A	43%	52%	Nisbet et al., 2019

Tci-mAPY-1** Tci-MEP-1						

Data not presented or not enumerated in manuscript
^Note, ovalbumin also gave a 55% reduction in this experiment when administered with the same adjuvant
*ND = not determined (ewes not euthanased)
**Loss of function mutant of Tci-APY-1

T.colubriformis

Table 5 provides a synopsis of vaccine trials with a range of antigens and preparations from *Tc*. From initial screens in the guinea pig model and the premise that surface or excretory-secretory (ES antigens) shed from various parasitic stages (L3-adult) would contribute to the solid protective immunity observed with trickle L3 infections (Dobson et al., 1990), L3 homogenates, post-exsheathment antigen (PEA) and native adult ES antigens were selected for analysis. Larval homogenates and soluble proteins, PEA and TcL3 pellets after extraction failed to generate consistent protection (>57%) when administered in a variety of adjuvants either subcutaneously (SC), intra-peritoneally (IP), inoculated into intestinal Peyers Patches (JPP/IPP) or when incorporated into cellulose gel and applied to the rectal mucosa (McClure, 2009; Table 2). Animals were consistently challenged with trickle infections of 1500-2000 *TcL3*, 3 times per week for 3 weeks to mimic natural infection. Throughout ongoing trials, antigens incorporated into alhydrogel (AH) generated higher levels of immunity than other adjuvants. Since this was the only adjuvant that did not elicit IFN-γ production on sheep (Emery et al., 1990), it was assumed that AH stimulated a Th-2 response, consistent with that providing conventional GIN immunity.

4.3.3.2 Excretory/Secretory (ES) antigens

T.colubriformis

Adult *Tc* produce around 1 mg of ES antigen per 25000 worms per day (unpublished observations). Early vaccine trials using either soluble proteins (SpL3) or ES antigens from *Tc*L3 or ES antigen from adult worms induced >90% reductions in worm counts in a guinea pig infection model (Rothwell & Love 1974). The level of protection was compromised significantly if ES proteins were administered in Freund's complete adjuvant as opposed to alhydrogel (Wagland et al., 1996). Subsequent comparisons in sheep were less successful, with the protection against worm burdens ranging from 30-70% when adult ES material (1mg/dose) was used (see Emery, 1996; Table 5).

T.circumcincta

Early vaccine trials used ES material from mixed stage *Telc* as an immunogen, administered in Freund's adjuvant to 9 month old cross bred lambs repeatedly over a period of 3 weeks (Table 4) (Rose, 1978). Three weeks after the final immunisation, the lambs were challenged by a trickle infection of 5,000 L3 daily for 15 days but no reductions in either FEC or worm count at post mortem were observed (Rose, 1978). In contrast, when 3 month old Dorset Horn lambs were immunised with concentrated ES material from mixed stage *Telc* (with or without Freund's adjuvant) and then

challenged by a short trickle infection of 7,500 L3 per day for 4 days, immunised lambs had between 48% and 69% fewer adult worms at post mortem and reductions in FEC of 47% to 60% when compared to unimmunised control lambs (Rose, 1978).

Other scour worms

Oesophagostomum radiatum

Homogenates of adult *O.radiatum* emulsified in Freunds complete adjuvant (FCA) reduced worm burdens of challenged calves (Keith & Bremner, 1973). Single intraperitoneal injections into cattle, of live *in-vitro*-grown L3 or L3-4 mixtures of *O.radiatum*, (Douvres, 1980) provided from 44 to 90% and 36 to 83% protection, respectively (Herlich et al., 1973). Two to three inocula of larval ES material (0.5-2mg), and semi-purified extracts from adult *O.radiatum* (cultured as per Douvres, 1983), given s/c in FCA or saline have reduced worm challenge burdens in calves by 99% and 88%, respectively (East et al 1988; 1993), but further research has not proceeded. Serious vaccine studies with *O.columbianum* have not been undertaken to date (Dhar & Singh, 1970).

Chabertia ovina. Vaccination trials have not been conducted although irradiation attenuates L3, causing self-limiting infections (Bezubik & Borowik, 1973; cited by Bain, 1999).

Nematodirus spp. Vaccination trials have indicated that 6 week-old lambs given a single dose of γ-irradiation attenuated larvae of *N. battus* had 66% fewer worms following challenge (Winter et al., 2000), but further studies in this and other *Nematodirus* species have not continued.

CarLA (**Car**bohydrate **L**arval **A**ntigen). Harrison et al (2003) described the isolation of a heat-stable 35 kDa carbohydrate antigen (CarLA) when TcL3 extracts were probed sera from with GIN-immune sheep and /or genetically resistant animals. Using antisera, the antigen was revealed to be shared by a range of GIN, including *Tc, Hc, Tel c* and *Nematodirus.* Binding was strongest to *Tc*L3 and less pronounced on *Hc*L3 and *Tel c*L3 (Harrison et al., 2008). CarLA was poorly immunogenic and a range of coupling agents were examined to attempt to boost its immunogenicity (W. Hein, pers. comm). Although the antigen was not tested directly as a vaccine (R.Shaw, pers. comm), indirect evidence of its putative protective capacity was inferred from the inhibition of establishment of *TcL3* which had been pre-incubated with immune mucus before dosing to sheep (Harrison et al., 2003). Interestingly, while anti-CarLA Igs in immune mucus inhibited *TcL3* establishment, *Hc*L3 and *Tel c*L3 were virtually unaffected (Harrison et al., 2008). It was later demonstrated that *Hc* at least, possessed different isoforms of CarLA, and that mucus isolated from sheep immunised against *Hc*L3 and *Tel c*L3 did not generate high levels of anti-CarLA antibodies (Harrison et al, 2008). However, titres of anti-CarRLA IgA, although transient, are indicative of worm immunity with genetic correlation around 0.6, and negatively correlate ($r^2 < 0.07$) with faecal worm egg counts (FWEC; Shaw et al., 2012).

Table 5: Results of Vaccine trials with *T.colubriformis* extracts and antigens

Extract/ antigen	**Dose**	**Route of vaccination**	**Adjuvant**	**% reduction (eggs)**	**% reduction (worms)**
Tc L3 Homogenate*	30000-50000	JPP/ rectal	Cellulose chitosan	49-53 10-25	30 10

	L3 *				
Post-exsheathment antigen (PEA)	100	IPP/IP	Quil/AH	34	48
	350	IPP/IP	AH	32	nd
Soluble Protein *Tc*L3*	5mg	rectal	Cellulose	0	0
	1mg	I/JPP	AH	12	10
*Tc*L3 pellet*	5mg	rectal	Cellulose	0	0
Adult ES antigens	100ug	IP	AH	52	47
ES 94kDa	50ug	IPP	AH	<30	<30
ES 11 kDa	50ug	IPP	AH	<30	<30
ES 11 +17kDa or +30kDa	100ug	IPP	AH	40	30-56
ES 30kDa	50ug	IPP	AH	<30	<30
ES recombinant 17kDa	250ug 100ug 33ug 10ug 3.3ug	SC x 2	Quil A	33 42 52 25 0	67 50 26 18 20
	100ug	IP x 2	AH Quil A IFA AH-Quil A	46 0 15 24	32 ns ns ns
	1mg	IPP x 2	Aqueous AH Quil A Dextran sulphate Alginate Fructose-1-P IFA Cellulose	34 43-62 42-53 66 39 57 41 37	3 24-57 26-41 37 28 13 19 47-71
	1mg	Rectal PP x 2	Cellulose Chitosan	<30 <30	<30 <30

	1mg	Pharyngeal LN x2	cellulose	0	0
ES recombinant 37kDa	20-50ug	SC	Quil A	<25	<25
	50ug	IP	Aqueous	ns	ns
			IFA	50	nd
			AH	46	nd
			Quil A	37-44	nd
			Dextran (DXS)	ns	nd
	100ug	IPP-mucosa	Dextran-AH	36	All
			IFA	16	<30
			AH	18	
		JPP-mucosa	Quil A	42	
			AH	11	
	500ug		PLG Microspheres	0	
	100ug	Rectal PP	Quil A	29	

Data from McClure (2009)* and Emery, McClure & Wagland (unpublished). Abbreviations include jejunal and ileal Peyers Patches (JPP/IPP), subcutaneous (SC) and intraperitoneal (IP) injection. Adjuvants include aluminium hydroxide (alhydrogel; AH), incomplete Freunds adjuvant (Montanide: Marcol 52; IFA), poly-lactide-glycolide (PLG) microspheres. IPP, JPP were direct injections during laparotomy while rectal and mucosal routes involved delivery of vaccine to the luminal surface of the epithelium (McClure 2000; 2008).

4.3.4 Recombinant antigen vaccines

The development of recombinant subunit vaccines for the protection of small ruminants against GIN has been, until recently, relatively unsuccessful (McClure, 2009; Nisbet et al., 2016a; Matthews et al., 2016; Stutzer et al., 2018). Recent work to develop a recombinant subunit vaccine for *Telc* has shown some promise in both lambs (Nisbet et al., 2013) and periparturient ewes (Nisbet et al., 2016b) and this is summarised below:

Teladorsagia circumcincta

Using a tripartite approach to identify antigens for inclusion in a T. circumcincta recombinant protein subunit vaccine, Nisbet et al., (2013) used previous evidence of the underlying immune mechanisms acting against the parasite (Smith et al. 1985, 1986, 1987), to select larval antigen targets of local IgA responses in sheep rendered immune to re-infection, focussing on L3 and L4 ES proteins. In addition, by bioinformatic analysis of stage-specific cDNA libraries (Nisbet et al., 2008), an immunogenic homologue of a protective antigen of the canine hookworm, Ancylostoma caninum [Ac-SAA-1 (Zhan et al., 2004; Nisbet et al., 2009)] was also identified. Finally, a suite of potentially immunosuppressive molecules released by the parasite was also identified (McSorley et al., 2010, Nisbet et al., 2010a; 2011). In total 8 potential vaccine antigens were identified by this process and

were prepared as a recombinant cocktail vaccine in Quil A. Experimental vaccine and challenge experiments (Table 3) were performed in Texel-cross 6-7 month-old lambs in two separate trials: In these studies, this prototype cocktail vaccine reduced cumulative faecal egg output on average by 70% and 58%, respectively, over a 6 or 10-week period after challenge (Nisbet et al., 2013). During the period of peak worm egg shedding, vaccinated lambs shed 92% and 73% fewer eggs than did Quil A adjuvant-only recipient lambs, respectively. At post mortem, vaccinates had 75% and 56% lower mean adult nematode burdens than adjuvant-only controls, respectively (Nisbet et al., 2013). In a subsequent experiment in lambing ewes, which displayed a periparturient relaxation in immunity, vaccination with this eight-protein cocktail resulted in a 44% reduction in mean cumulative faecal egg count levels in recipient ewes compared to adjuvant-only control animals (Nisbet et al., 2016b). More recently (Nisbet et al., 2019 in press), using data from five independent vaccine trials, which employed the eight-protein subunit cocktail vaccine, a strategy was developed to simplify antigen complexity of the vaccine. A meta-analysis of data from these five trials demonstrated statistically significant reductions in FEC and worm burden in vaccinated sheep when compared to those which had received adjuvant only ($p = 0.009$ and $p < 0.0001$, respectively). Relationships between antigen-specific antibody levels, antibody avidity and parasitological parameters of efficacy were analysed for each of the eight proteins in these trials. Of these, the strongest correlations between percentage reduction in cFEC and avidity were obtained for the vaccine antigen *T. circumcincta* apyrase-1 (Tci-APY-1) in relation to either total antigen-specific IgG or IgG1 in sera. In addition, IgG and IgA within the serum and abomasal mucus of control (parasite challenged) lambs strongly recognised Tci-APY-1 and *T. circumcincta* metalloproteinase-1 (Tci-MEP-1) but only weakly bound the other six antigens, indicating Tci-APY-1 and Tci-MEP-1 are most effectively recognised by the parasite-induced antibody response. On the basis of these findings, a two-protein vaccine comprising Tci-APY-1 and Tci-MEP-1 was tested in a direct comparison with the original eight-component vaccine (Nisbet et al., 2019). A further group was immunised with Tci-MEP-1 in combination with a mutated form of Tci-APY-1 (mTci-APY-1), which had no enzymatic activity. Across the trial, the mean FEC levels of the eight-antigen recipients were lower than those of the adjuvant only control group ($p = 0.013$) and the mean FEC of the mTci-APY-1 and Tci-MEP-1 recipients was lower, though not statistically significantly, than that of the adjuvant-only control group ($p = 0.093$). Mean cFEC levels were reduced by 43% in lambs immunised with mTci-APY-1 plus Tci-MEP-1 compared to the controls ($p = 0.079$) (Nisbet et al., 2019).

Haemonchus contortus

Several groups over recent decades have attempted, without success, to emulate the vaccine efficacy of native Haemonchus gut membrane glycoproteins by producing components of the H-gal-GP complex or H11 as recombinant proteins in a variety of prokaryotic and eukaryotic expression systems and testing these individually or in combination in protection studies (summarised in Nisbet et al., 2016a).

Recombinant versions of other Haemonchus vaccine candidates have also met with variable, or limited, success. Protection studies with combined native 15 and 24 kDa E/S proteins gave substantial levels of protection in 8 month old Texel sheep (Schallig et al., 1997) and bacterially-expressed recombinant versions of both proteins initially gave promising results when combined and co-administered to 7-9 month old sheep resulting in 38-42% reduction in FEC and 55-65% reduction in worm burden compared to the adjuvant-only control (Vervelde et al., 2002). However,

subsequent attempts to repeat these results in 3 month old lambs and in 9 month old sheep did not result in reductions in faecal egg count or worm burden (Vervelde et al., 2002). A bacterially-expressed recombinant form of the *H. contortus* enolase (*Hc*ENO) administered as a water-in-oil emulsion with the adjuvant Montanide ISA 61 VG, was effective in halving both FWEC and worm burden in vaccination/challenge trials in 6 month old Mecheri breed lambs (Kalyanasundaram et al., 2015). Bacterially-expressed recombinant versions of two isoforms of a putative immunomodulatory galectin from *Hc*, termed *Hco*-gal-m and *Hco*-gal-f induced partial protection in vaccine trials in 9-10 month old goats (37-48% reduction in FWEC, 41-46% reduction in worm burden) when administered in Freund's adjuvant (Yanming et al., 2007). When 3 major cathepsin B-like cysteine proteinases - hmcp1, 4, and 6 were expressed in *E.coli* as GST-fusion recombinant proteins and used to immunise Suffolk/Greyface cross sheep, a cocktail of the three proteins conferred partial protection, with reductions in worm burden of up to 38% (Redmond & Knox, 2004). When the same cocktail of recombinant proteins was used, but without fusion partners on the recombinant proteins, reductions of 27% and 29% in faecal egg counts and worm burdens were observed, respectively (Redmond & Knox, 2006). One of the more promising antigens identified in the last decade for exploitation as a recombinant vaccine against *Hc* is the somatic antigen Hc23, an exposed antigen of unknown function which constitutes ~1.8% of the total aqueous somatic extract of adult *Hc* (Fawzi et al., 2014). Pilot studies with the native protein suggested that *Hc*23 may be used as an effective vaccine antigen when injected into 4-5 month old lambs: Reductions in faecal egg counts and abomasal worm burdens were 70% and 67% respectively when native Hc23 was co-administered with aluminium hydroxide; 85% and 87% respectively when co-administered with bacterial lipopolysaccharide/inactivated bacteria as adjuvant (Fawzi et al., 2014). It should be noted, however, that these values relate to reductions when compared with an un-immunised challenge group rather than control groups which had been administered the appropriate adjuvant only (Fawzi et al., 2014). A bacterially-expressed recombinant form of *Hc*23 administered with AH, was also effective in reducing FWEC and worm burden (by 83% and 85% respectively, Table 1) in vaccination/challenge trials in 4-5 month-old Assaf breed lambs (Fawzi et al., 2015). Again this trial suffered from a lack of appropriate, adjuvant-immunised only, controls but offers some hope for the use of recombinant proteins as vaccines to control haemonchosis.

Trichostrongylus colubriformis

Several adult ES proteins expressed in E.coli and baculovirus recombinant forms (11, 17, 30, 37 & 94 kDa) have been investigated as protective immunogens in sheep (McClure, 2008; Table 5). An exhaustive series of trials in sheep utilising adjuvant combinations and targeting mucosal routes (IP, Peyer's Patches and rectal mucosa) of delivery to mimic induction of conventional immunity, sensitise mucosal mast cells (MMC) and generate worm-specific IgG1, IgA and IgE were undertaken (Emery, 1996; McClure, 2008). Table 4 lists much unpublished work to prevent repetition of past efforts. Overall, these antigens and protocols failed to generate repeated levels of >70% protection to be pursued further. However, there was little doubt that mucosal vaccination, especially through IPP and JPP using antigens in AH effectively generated antigen-specific IgG1, IgA and IgE responses. Overall, the exact mechanisms by which GIN interact with the sheep mucosal immune system to elicit the slow development of immunity (compared to anti-bacterial responses) are unclear, but the ultimate outcome of a Th2-type response is well documented.

4.4 Novel expression systems

While technologies to identify parasite genes and transcripts have progressed rapidly, a major hurdle in vaccine development is expression of vaccine candidate genes in appropriate expression systems. *Eschericia coli* or *Pichia* may be suitable for some proteins (Nisbet et al., 2013), however others will require more complex systems. Genes from *Telc* and *Hc* can be expressed in *C. elegans* and can rescue *C. elegans* mutant phenotypes, demonstrating correct folding and functional activity (Britton & Murray, 2006; Baker et al., 2012). While *C. elegans* may be appropriate for expression of some parasite proteins, we have been unable to express *Hc* vaccine antigen H11 or cysteine or aspartyl proteases in *C. elegans* in a form that can induce protection against challenge infection (Roberts et al., 2013). There are several possible reasons for this and may indicate that a combination of antigens is required to induce a protective immune response. Alternatively, specific glycans present on H11 and other vaccine antigens may be required to induce protective immunity and these glycans are not present on *C. elegans* expressed proteins. Detailed mass spectrometry comparison of glycans present on native *Hc* H11 and *C. elegans*-expressed recombinant H11 identified similarities but also some differences: similar di- and trifucoslyated glycans were identified but there was a lack of fucosylated LacdiNAc (LDNF) and Galα1-3GalNAc structures on *C. elegans* recombinant H11 compared to the native protein (Roberts et al., 2013). The use of synthetic glycans as vaccines would help test the relevance of glycan structures to protection (Astronomo & Burton, 2010).

Where glycans and other post-translational modifications introduce conformational epitopic structures to the protective antigens and these can be extremely challenging to reproduce in a recombinant expression system. One approach is to use commercially-available random peptide phage-display libraries to identify short peptide epitopes that are bound either by antibodies in sera from animals protected against challenge by prior immunization or by previous infection. These phage-displayed peptides may represent primary protein sequence or more complex secondary or tertiary structural epitopes and may even mimic non-peptide components such as carbohydrate moieties. Phage-displayed peptides have been used in the development of prototype vaccines against a range of parasites including *Trichinella spiralis* (reviewed in Ellis et al., 2012). Preliminary work using antibodies from sheep rendered immune to infection with *Telc* by trickle infection to pan phage-displayed peptide libraries identified 5 peptide sequences which mimicked surface and/or glycan epitopes on exsheathed L3 larvae (Ellis, 2014). Positive correlations were demonstrated between peptide-specific IgA levels for these 5 peptides and the percentage of inhibited L4 larvae present in the abomasum, whereas there were negative correlations between the levels of peptide–specific IgA and total nematode burden (Ellis, 2014).

Plant expression systems are being more widely tested for expression of parasitic nematode proteins, particularly those where folding and/or glycosylation are critical for function. Plant expression systems provide a number of benefits, including the production of high levels of protein in soluble, secreted form and technology to manipulate their glycosylation machinery (Wilbers et al., 2017). Transgenic tobacco plants have been used in recent studies to determine the structure and binding properties of nematode venom allergen-like proteins (VALs) (Asojo et al., 2018; Darwiche et al., 2018). A similar approach would be valid for testing expression, function and protective capacity of related activation associated proteins (ASPs) and other vaccine candidates from scour worms.

5 Technological advances and opportunities (and how they can be used)

Introduction. Current status of worm assays.

As detailed below, there are limitations for the *in vitro* cultivation and maintenance of sheep GIN. While exsheathed *Hc*L3 readily develop to L4 in simple medium, much more complex conditions are needed for development of both *Hc*L3 and *Tc*L3 to adult parasites (Douvres, 1980; Stringfellow, 1986; P.Presidente, unpublished), limiting use for widespread analysis of developmental-specific processes, pathways and screening. Consequently, researchers have relied on supplies of infective L3 (now limited in Australia), or parasites at various stages of development sampled directly *ex vivo* (eg. Rowe et al., 2005; McNeilly at al., 2017), with outcomes measured variously as viability (survival), egg production or comparative measurements of length or sex ratios (e.g. Rowe et al., 2005; McNeilly et al., 2017). Studies on immunity as well as high-throughput screens for new anthelmintics have relied primarily on the Larval Development Assay (LDA; Lacey et al., 1990) or paralysis of L3 motility in various "wriggle-ometers", with or without downstream 'omics investigations (Jones et al., 1994; Hu et al., 2013). Other investigations of RNAi gene silencing and functional validation have utilised short-term cultures, with assay of the target gene, its transcription or products (Kotze & Bagnall, 2006) or worm survival *in vivo* (Samarasinghe et al., 2011).

Greater sophistication of culture methods has occurred with the movement from abomasal explants (Jackson et al., 2004) to intestinal organoids (Powell & Behnke, 2017; Hamilton et al., 2018) or cultured gut cells (N. Andronicos, *pers. comm*. 2019) that may support GI nematode development for antigen identification, metabolic profiling and target screening. Progress in this area would greatly assist vaccine development and antigen validation.

5.1 Worm genome progress for antigen identification

Vaccine candidate identification has relied mainly on the use of antisera from immune or convalescent animals to screen either cDNA libraries or immunoblots for immunodominant antigens (e.g. Verkuylen et al., 1993; Redmond et al., 2006) or in immunoaffinity purification (e.g. Ellis et al., 2014). However, over the last decade information on the complete set of genes present in parasitic nematodes, the lifecycle stages in which these genes are expressed and detail of their putative functions, has greatly increased our knowledge of parasite biology. This establishes a platform for rational selection of potential vaccine targets. These may include enzymes catalyzing unique metabolic pathways (chokepoints), secreted and surface proteins, and molecules with immunoregulatory properties. Importantly, detail of the genomic composition of more and more species will help establish a map of genes conserved across diverse nematodes and those specific to individual nematode clades or species. This information can help identify biological function and reveal potential pan-nematode targets, relevant for commercial vaccine development.

5.2 Antigen identification from genomic and transcriptomic data

5.2.1 Genomics and transcriptomics

Genome data is available for a wide range of nematode species, including many of veterinary importance. A comparative genomics analysis of 56 nematode species, including *Hc* and *Telc*, has

recently been published (International Helminth Genomes Consortium, 2019), which has been supported by the 50 Helminth Genomes project, a collaboration between The Wellcome Sanger Institute (WSI, UK), Washington University, USA and Edinburgh Genomics, UK (https://www.sanger.ac.uk/science/collaboration/50hgp). Progress has been helped by the reduced cost of sequencing, together with improved sequencing technologies and bioinformatics pipelines. It is therefore feasible to sequence the genome of GI nematodes relatively quickly, although generating high quality assembled and annotated genome data is still challenging. This is due to the large size of GI nematode genomes (approximately 300-700Mb, representing an estimated 20,000 protein coding genes) and the high level of polymorphism that exists between individual parasites within a population. Of the GI nematodes of veterinary importance, most advanced genome information is currently available for *Hc*, sequenced independently from UK and Australian isolates (Laing et al., 2013; Schwarz et al., 2013). This was achieved using Roche 454 and Illumina sequencing technologies, which provide short DNA reads from both ends of DNA insert libraries. Overlapping sequences can then be assembled into contigs and scaffolded by mate pair data. A first draft genome for *Hc* was completed in 2013, however refinement of this initial genome assembly has been ongoing at WSI. This has involved the use of PacBio long-read sequencing, which produces average read lengths of 30kb, optical mapping and intensive manual curation, enabling assembly of the *Hc* genome at a chromosome level (Doyle et al., 2017). This assembly has been annotated with the inclusion of Iso-Seq read data, which identifies full-length cDNA sequences.

The technologies applied to *Hc* genome sequencing, assembly and annotation have provided a highly relevant model applicable to related GI nematodes, including *Telc* and *Trichostrongylus* species. *Telc* genome sequencing and assembly are in progress at WSI, UK. In common with *Hc*, the extensive genetic diversity in the parasite population and large genome size are presenting challenges to reliable sequence assembly. The genome of *Telc* is strikingly larger than that of other nematodes (S Doyle and J Cotton, unpublished data). Genetic polymorphism was previously observed for *Hc* and has arisen due to natural variation within the large parasite population and the mating of females with multiple males (polyandry), a phenomenon also reported for *Telc* (Redman et al., 2008; Gilleard & Redman, 2016). Novel approaches, such as single cell sequencing technology, are being investigated at WSI to aid *Telc* genome assembly (S Doyle, R Laing & J Cotton). For *Tc,* genome sequencing is in progress at Washington University (see https://www.ars.usda.gov/research/project/?accnNo=431526). Available sequence data for parasite genomes can be accessed at the WormBase ParaSite website (https://parasite.wormbase.org/index.html), though of the worms of importance to the Australian sheep industry, only *Telc* and *Hc* appear in this database. WormBase ParaSite allows retrieval and comparison of gene sequences across nematode species, as well as predicted functional information based on gene ontology (GO) data. Genome information for *H. contortus* has identified enrichment for specific types of genes, including those for proteolysis, neurotransmission and carbohydrate metabolism (Laing et al., 2013). However, the function of many nematode genes is currently unknown; it is notable that 47% of nematode gene families lack any functional annotation, indicating their novelty (International Helminth Genomes Consortium, 2019). Availability of genome data for *Telc* and *Trichostrongylus spp* will allow identification of genes and pathways conserved across GI nematodes, that differ to those of the host, and that may be rationally selected as potential vaccine candidates.

In comparison to genomic data, transcriptomic data have traditionally been more readily acquired and can be used in vaccine candidate identification to inform protein identification from immunoblots, immunoaffinity purification, by homology to known vaccine candidates in other species or through "reverse vaccinology" (see below) and rational selection. Transcriptomic data are publically-available for some species of scour worms and these data are usually generated from the parasites at specific developmental stages, living in specific niches or in some cases, from individual sexes. For *Tc*, transcriptomic data for adult (mixed sex) worms was generated by Roche 454 sequencing (Cantacessi et al., 2010) and these data, which represent ~22,000 contigs, have been deposited in publically-accessible sites (http://www.nematode.net/ and http://research.vet.unimelb.edu.au/gasserlab/index.html). This represents the most comprehensive set of transcriptomic data for this species. In contrast, a current (Oct 2018) search of the NCBI nucleotide (non-redundant) database for gene sequences from *Tc* yields only 259 sequences, the vast majority of which are for internal transcribed spacer sequences from various isolates or beta tubulin sequences, a notable exception being the "*Trichostrongylus colubriformis* globin-like host-protective antigen" deposited by Frenkel et al., in 1993. Stage-specific transcriptomic data are currently absent from public databases for *Tc*. For *T. vitrinus* there is even less publically-available transcriptomic sequence; studies by Nagaraj et al. (2008) and Nisbet et al. (2004) used the same set of sex-specific adult expressed sequence tags (ESTs), generated by suppressive-subtractive hybridisation (SSH) and these studies resulted in the deposition of 376 ESTs for this species in the NCBI databank. SSH libraries are constructed from restriction enzyme-digested cDNA and, as such, are often composed of short, non-overlapping fragments of cDNA which may limit the value of ESTs produced by this process for other downstream applications, (e.g. protein identification from translated EST data). In total, 383 ESTs and 53 nucleotide sequences currently exist in the NCBI databank for *T. vitrinus*. For other species of *Trichostrongylus* there are even fewer publically-available nucleotide resources to inform vaccine development. In contrast to the paucity of EST data for some *Trichostrongylus* species, there is a reasonable quantity of transcriptomic data publically available for *Telc*: Menon et al. (2012) assembled 407,353 raw sequences (NCBI EST database accession numbers SRR328404 and SRR328405) derived from Roche 454 sequencing of adult stage cDNA libraries into 24,013 protein-encoding contig sequences. Stage-specific (exsheathed L3 and L4) libraries generated by SSH contributed 1,744 ESTs (Nisbet et al., 2008) and, in NEMBASE (http://www.nematodes.org/) there are 6,011 ESTs derived from L3, L4 and adult worms. Currently on http://nematode.net there are 33,148 isotigs available (derived from Roche 454 sequencing) representing 25,567 genes for which RNAseq expression data are available. Additional, "niche-specific" transcriptomes, also exist for *Telc:* Halliday et al. (2012) produced 6,969 clusters of isotigs from exsheathed L3 *Telc* and demonstrated differential expression of a set of 18 isotigs in larvae exposed to abomasal extracts from immune and non-immune sheep. More recently, McNeilly et al. (2017) compared the transcriptomes of two fourth-stage larval populations of *Telc*, which differed in their location within the abomasum. These were either mucosal-dwelling (MD) or lumen-dwelling (LD) and 57 transcripts were identified which were significantly differentially expressed between MD and LD larvae. Of these transcripts, the majority (54/57) were up-regulated in MD larvae and encoded a suite of potentially immune-regulatory proteins produced by the parasite only when in intimate contact with the host which may be exploitable as vaccine candidates (McNeilly and Nisbet, 2014).

For *Hc*, a wealth of transcriptomic data has accompanied the genome sequencing projects and RNAseq expression data are publically available (Laing et al., 2013). Tissue specific transcriptomics has also accompanied the publication of the *Hc* genome and, of particular relevance to vaccine development, a female gut-specific transcriptome was generated by comparing the female gut expressed mRNAs with those from the whole female worm (Laing et al., 2013). This identified a suite of gut-expressed cysteine-type peptidase and cysteine-type peptidase inhibitors. Recent work on differential transcriptomic analysis in *Hc* has also demonstrated that worms surviving in sheep which had been vaccinated with Barbervax® had an increased expression of genes encoding (non-Barbervax®) proteases and regulators of lysosome trafficking (Sallé et al., 2018) compared to worms from un-vaccinated animals. Both of these pieces of work add weight to the previous research suggesting that gut proteinases, and their regulators, are strong vaccine candidates in this and other species (reviewed in Nisbet et al., 2016). Normal text

5.2.2 Reverse vaccinology

The traditional design of vaccines, by isolating, cultivating and inactivating pathogens was complemented (rather than being entirely replaced) by the process of “reverse vaccinology” in the mid-late 1990’s following the advent of whole genome sequencing (WGS) of pathogens (Figure 5). Thus, the first publication of a whole bacterial genome (from *Haemophilus influenzae*) in 1995 (Fleischmann et al., 1995) was followed, in 2000, by the use of a whole bacterial genome sequence to identify vaccine candidates for serogroup B meningococcus (Pizza et al., 2000) and the causal organism of ovine footrot (*Dichelobacter nodosus*; Myers et al., 2007). Ultimately this led to the licensing and use of a vaccine for use against meningococcus (Nolan et al., 2015), but lack of protective success against footrot so far (Goding & Dhungyel, unpublished). The original reverse vaccinology approach used genomics coupled with high throughput recombinant protein expression and finally, screening *in vivo* for early antigen discovery. This process has since evolved in “reverse vaccinology 2.0” which adds the development of host-specific monoclonal antibody (MAbs) production, B cell repertoire deep sequencing, proteomics and structure-based antigen design to the original approach (reviewed in Rappuoli et al., 2016).

In reverse vaccinology, vaccine candidates are initially identified using widely available *in silico* algorithms (e.g. Vaxign, http://www.violinet.org/vaxign/ He et al., 2010; current resources recently reviewed in Lew-Tabor & Valle, 2016) to predict open reading frames of surface or secreted proteins from all proteins inferred from a whole genome analysis. For this reason, a complete, assembled, annotated genome is the preferred starting point, though a deep stage-specific transcriptomic analysis in the case of vaccines targeting specific developmental stages of parasitic nematodes, for example, might be used as a reasonable proxy. These genomic/transcriptomic components of the classical reverse vaccinology approach are either already available (e.g. in the case of *Hc* with a full published genome), are in development (*Telc, Tc*) or are lacking (*T. vitrinus*) (recently reviewed by Jex et al., 2019). The other components of classical reverse vaccinology - capacity to produce recombinant proteins in high throughput and robust animal models for the testing of candidate antigens - are both available for testing of prototype vaccines against scour worms in a small number of Universities and Research Institutes internationally. However, the large genomes of parasitic helminths (in comparison to viral and bacterial genomes) and the potential that recombinant vaccine candidates may need to be expressed in eukaryotic systems for optimal efficacy both make the traditional reverse vaccinology route cumbersome for parasitic worms (Merrifield et al., 2016).

Nevertheless, significant progress has been made in the use of reverse vaccinology to identify vaccine antigens from the parasitic helminth *Schistosoma mansoni* through the *in silico* prediction of immunogenic epitopes of transmembrane proteins coupled with selection of these epitopes for high binding affinities for host MHCII molecules (de Souza et al., 2018; Oliveira et al., 2016).

In sheep, the MHC class II molecules that most strongly determine antibody specificity are encoded by seven loci: *DRB1, DQA1, DQB1, DQA2, DQB2, DQA3* and *DQB3*. Alleles at these loci can be readily detected by DNA sequencing. The *DRB1*1101* molecule has been associated with reduced faecal nematode egg counts counts in sheep of the Scottish Blackface (Stear et al. 2005), Suffolk (Sayers et al. 2005) and Texel breeds (in preparation). Further this allele has been associated with reduced worm numbers in both natural (Stear et al. 2006) and deliberate infection (Hassan et al. 2011). Various authors have suggested using epitopes with high binding affinities to MHC molecules as potential vaccine candidates (eg. de Souza et al. 2018). However, there are several reasons why this approach is unlikely to be successful in sheep. Firstly, MHC-mediated resistance probably acts through IgE activity and IgE is associated with increased pathology (Stear et al. 2003). Secondly, resistance to natural infection involves the recognition of multiple molecules (Murphy et al. 2010); animals that are homozygous for the resistance allele *DRB1*1101* show increased susceptibility to nematode infection and the mechanism underlying MHC-mediated resistance is the recognition of a better set of parasite molecules in heterozygotes. In other words, the MHC does not provide a short cut to identifying highly protective candidate antigens. Algorithms developed for human MHC molecules can potentially help identify immunogenic molecules from sheep parasites but standard proteomic approaches (Ellis et al. 2014; Murphy et al. 2010) have already identified over 150 immunogenic molecules from *Telc* L3 that are immunogenic in sheep.

Recently, significant progress has been made in the use of reverse vaccinology approaches to develop vaccines against the cattle tick (*Rhipicephalus microplus australis*) and the Australian paralysis tick (*Ixodes holocyclus*) (Lew-Tabor et al., 2016; Tabor, 2019) with a vaccine for the former having gone through 12 years of antigen discovery, bioinformatics, *in vitro* immune screening and *in vitro* tick feeding followed by seven proof of concept cattle tick challenge trials. Ultimately, with funding from Meat and Livestock Australia (MLA project code B.AHE.0212, report published October 2017) amongst others, this has led to the discovery of 6 antigens for which a patent has been filed in February 2018 and commercial exploitation is imminent.

Reverse vaccinology 2.0 is considerably more advanced in the field of human vaccine development than in veterinary species. Because most licensed vaccines act by inducing protective antibody responses, most of the key breakthroughs for reverse vaccinology 2.0 have revolved around antibody technologies (“antibodyomics”). The key elements of the approach that have greatly enhanced vaccine design for human medicine in recent years (reviewed in Rappuoli et al., 2016) have been:

- The development of recombinant mAbs or their antigen binding fragments from cloned B cells derived from immunised (protected) or convalescent (immune) individuals. These can then be used to screen for their neutralising ability against the pathogen (requiring an *in vitro* assay, see section 6.5, below) and to identify the specific epitopes that they bind (see section 6.2.3, below)

- High throughput, deep sequencing transcriptomic analyses to analyse the B cell repertoire, understand antibody maturation following infection or immunisation and devise strategies to prime B cell precursors expressing germline-encoded antibodies.
- Mapping of conformational epitopes through 3-D analysis of their interactions with the antigen binding fragments of neutralising antibodies.
- New computational approaches to understanding the structural and immunological data to design novel vaccines to stimulate specific immunological responses to protective epitopes.

5.2.3 Epitope mapping

The likely requirement for multiple recombinant antigens to be co-administered to effectively control complex eukaryotic parasitic organisms by vaccination (e.g. see Makepeace et al., 2009; Nisbet et al., 2013) makes the antigen discovery, production and commercialization processes challenging. A potential solution to the latter two issues is to map the most immunogenic epitopes of individual antigens and then to administer these as concatemerised peptides. An additional benefit of this approach may be that smaller epitopes are more likely to be conserved across nematode species so this may offer an approach to pan-species vaccination. Using web-based tools, B and T-cell epitopes can be predicted *in silico* from the protein sequence (inferred from transcriptomic or genomic data) of potential vaccine antigens (e.g. the Immune Epitope Database and Analysis Resource server at https://www.iedb.org/ and/or the NetCTL server http://www.cbs.dtu.dk/services/NetCTL/). Empirical evidence to underpin the *in silico* epitope mapping of linear B cell epitopes can be generated using immune or convalescent sera to probe overlapping peptides representing the entire protein either on peptide arrays or in ELISA plate format (e.g. see Dobrut et al., 2018) whereas T cell epitope mapping can be achieved using peptide libraries coupled with ELISpot assays (e.g. see Zhao et al., 2018).

An epitope mapping strategy is being pursued for parasitic organisms (e.g. *Trypanosoma cruzi;* Khatoon et al., 2018) where immunogenic epitopes of secretory and membrane proteins of the parasite were predicted and produced as a synthetic construct along with molecular docking analysis to optimize the interaction between TLR-2 and TLR-4 receptors and the synthetic vaccine. In parasitic nematodes, a recent publication by Immanuel et al. (2017) described the design, synthesis, and efficacy testing of short peptide epitopes from thioredoxin and transglutaminase of filarial nematodes constructed as a “multiple antigen peptide” on an inert lysine core. In vaccine efficacy tests using the jird model, the synthetic vaccine produced high levels of antigen-specific antibody, a cellular response and significant levels of protection (Immanuel et al., 2017).

5.2.4 Immune profiling

Historically, many candidate antigens for vaccine trials were differentially identified using serological reagents from small numbers of immune vs susceptible sheep (genetically selected or outbred) (Stear et al., 1995; Emery 1996; Nisbet et al., 2016). However, helminths produce thousands of molecules but only a small proportion are accessible to the immune system and an even smaller proportion are capable of generating protective immune responses. The major mechanisms of immunity against GIN are the IgE mediated regulation of parasite numbers (*Tc* and *Telc*) (Bendixsen et al., 1995) and the IgA-mediated control of parasite growth and fecundity (Stear et al. 1995). The

target for protective IgE responses is third-stage larvae, and ES extracts with major allergens can be identified by ELISA and immunoblotting (Murphy et al., 2010) or degranulation of mucosal mast cells (Bendixsen et al., 2004). In *Telc*, IgA acts against fourth-stage larvae and some of the nematode molecules recognised by IgA have been identified by immunoblotting (Redmond et al., 2006; Smith et al., 2009) and used in a recombinant vaccine against *Telc* (Nisbet et al., 2013). In addition, Ellis et al. (2014) identified a suite of 155 IgA-reactive proteins from L3 *Telc* by immunoaffinity chromatography and levels of immunoaffinity-enriched L3 antigen-specific IgA in gastric lymph from previously-infected sheep were significantly higher than those measured in helminth-free sheep. In the same study, there was a significant negative correlation between immunoaffinity-enriched L3 antigen-specific IgA levels in efferent gastric lymph and total *Telc* burden measured at necropsy and a significant positive correlation between immunoaffinity-enriched L3 antigen-specific IgA levels in efferent gastric lymph and the percentage of inhibited L4s enumerated at necropsy (Ellis et al., 2014). Taken together, this suggests an involvement of L3-specific IgA in some aspects of immunity against *Telc*.

An expanded approach for immune profiling for *Telc*, which would then be adopted for each of the important scour worm species (summarised in Figure 6, Section 7), would be to start with a comprehensive annotated genome, stage-specific transcriptome, proteome and glycome from the parasite to inform all downstream analyses. The downstream analyses would employ immunoaffinity chromatography to separate immunoreactive parasite molecules from each parasitic stage followed by mass spectrometry to identify immunoreactive proteins and glycans within these molecules (as described in Ellis et al., 2014). Mass spectrometry data would then be used to search the genomic data that we and others have sequenced to identify genetic variants in these molecules. An additional downstream approach would be to generate high-density peptide microarrays, which can now represent up to 2 million pre-addressable peptides on a single chip (Hansen et al. 2017; Østerbye et al. 2015). Peptides would be selected using in silico reverse vaccinology approaches to represent selected open-reading frames within the parasite genomes and arrays screened with antibody from immune protected sheep to precisely define potentially protective linear B cell epitopes (and by extension antigens targeted by CD4+ T cell responses. A similar approach can also be taken to interrogate glycan antibody reactivity in which microarrays consisting of glycans isolated from different parasite life-stages are used for antibody screening (Yang et al. 2018). Researchers could then develop more-focussed, molecule-specific ELISA, peptide and glycan arrays to quantify the response to each molecule by naturally and deliberately infected sheep with ranked FWEC. Sophisticated statistical methods, especially generalized linear mixed modelling, will rank molecules by their influence on resistance to infection. This immune profiling has been used by us and others, especially in the study of human malaria. It provides a way to identify those molecules which generate protective immune responses. To add power and greater certainty to rank potential vaccine antigens, recent advances have enabled immune profiling of parasite extracts using reagents from a much larger numbers of animals. Animals proposed for immune profiling would need to have their worm resistance status verified by FWEC or similar measures, but may come from biobanks, resource flocks, the Merino Lifetime Performance (MLP) project or from deliberate trials.

5.3 Antigen Validation by Gene silencing

While new ‘omics data can help select and narrow down the list of vaccine candidates, validation of these is required for any vaccine development pipeline. Ideally, this would involve gene knockdown

and analysis of resulting phenotypes (e.g. larval arrest, lethality) *in vitro*, reducing the ethical and cost concerns of *in vivo* testing. Technologies to test the essential function of target proteins are available. However, optimization is required to apply these to most nematodes. RNA interference (RNAi), whereby exogenously delivered double-stranded RNA (dsRNA) targets the corresponding mRNA for degradation, has been shown to function very effectively in the model nematode *C. elegans*. Silencing of *C. elegans* transcripts can be achieved by soaking worms in dsRNA, feeding on bacteria expressing dsRNA or microinjection of dsRNA. However, in other nematode species, soaking and feeding are far less effective (Felix, 2008). Using various delivery methods, RNAi has been shown to silence some genes in GI nematodes, including *Hc* (Kotze & Bagnall, 2006; Geldhof et al., 2006), *Telc* (Tzelos et al., 2015) and *Tc* (Issa et al., 2005) (Table 6). By targeting a range of genes, those expressed in the parasite intestine, amphids and excretory system could be silenced, while others were refractory (Samarasinghe et al., 2011). This indicated that RNAi is useful for testing the essential function of genes expressed in sites accessible to the trigger dsRNA but, using current technology, is not suitable for high-throughput functional genomics. RNAi of the H11 gene in *Hc*L3 *in vitro* prior to infection resulted in a 57% reduction in FWEC, 40% reduction in worm burden *in vivo* and 64% decrease in aminopeptidase activity compared with pre-soaking L3 in control dsRNA (Samarasinghe et al., 2011). This provided proof of principle that RNAi could be used for target selection. The RNAi pathway is therefore functional in GI nematodes but improvements to the reliability and robustness of silencing and phenotypic or proteomic analysis are needed for this to be applied as a potential vaccine-screening platform (Geldhof et al., 2007; Britton et al., 2016).

Stable transfection of nematodes with viral vectors expressing dsRNA to parasite genes is one route to improve on the delivery and persistence of RNAi. Transduction with lentiviral constructs expressing short hairpin RNAs has been shown to induce specific gene silencing and phenotypic effects in *S. mansoni* eggs (Hagen et al., 2014). Similar technology is being investigated for the mouse GI nematode *Nippostrongylus brasiliensis* (Hagen and Selkirk, unpublished data; https://gtr.ukri.org/projects?ref=BB%2FS001085%2F1). This has clear relevance for progressing RNAi gene silencing for scour worms.

An alternative approach that is being widely applied in mammalian systems and *C. elegans* is the use of CRISPR/Cas9 gene editing. This involves the introduction of single guide RNA designed to a specific target DNA sequence; this directs the Cas9 nuclease to create double-stranded breaks within the genome at the target sequence site. In *C. elegans,* CRISPR/Cas9 gene knockout is highly efficient and specific (Chen et al., 2013). Recently, this technology was successfully applied to the human GI parasite *Strongyloides stercoralis* (Lok et al., 2017; Gang et al., 2017). Efficient delivery of the guide RNA and Cas9 gene/protein are the main hurdles to overcome for this to be adopted more widely in parasitic nematodes. *Strongyloides* species have a free-living cycle and free-living adult female worms can be transfected by microinjection in a similar manner to *C. elegans* (Lok et al., 2017). Effects of CRISPR/Cas9 gene silencing are then examined in the F1 generation following male mating. Importantly, the findings of Gang et al. (2017) suggest that in *S. stercoralis*, homozygous gene deletion occurred and was detectable in the F1 generation, without the need for further parasite passage. Although microinjection of individual worms is not a high-throughput approach and not applicable to all nematodes, the use of viral vectors or nanoparticles to deliver CRISPR/Cas9 (reviewed in Lino et al., 2018), combined with improved *in vitro* culture systems for parasite maintenance (see below), may provide a route for robust gene silencing in veterinary GI nematodes. Such an approach could be readily applied to validate important genes which regulate stage-specific

development or metabolism in *ex vivo* studies and which may be amenable to vaccine –induced neutralisation.

5.4 Translational silencing

A novel methodology using peptide conjugated phosphorodiamidate morpholino oligomers (PPMOs) has also emerged for the analysis of protein function and to determine the phenotype resulting from protein knockdown. PPMOs are non-ionic DNA analogues attached at the 3'-end to a peptide which allows transmembrane movement (Li and Morcos, 2008). Translation of a specific target mRNA in the cell or organism is blocked through Watson/Crick base-pairing of the PPMO with the 5′ -UTR through to 25 nucleotides downstream of the initiation codon of the target mRNA, physically preventing the assembly of the ribosome at the initiation codon (Summerton, 1999). This post-transcriptional silencing allows assessment of whether these proteins are essential for life and are therefore good targets for drugs or vaccines. PPMO-mediated translational silencing may be a promising alternative to RNAi which has had limited success in parasitic nematodes. Recently, Witola et al., (2016) used this technique to silence phosphoethanolamine methyltransferase expression in adult *Hc in vitro*, resulting in a measurable phenotype. Several components are required for successfully carrying out PPMO-mediated silencing:

- Transcriptomic knowledge to ensure that the target mRNA is expressed in the stage being investigated and that the sequence of the PPMO oligomer is unique to the target gene.
- A method to measure the knockdown of the protein (quantitative immunoblotting, qProteomics, enzyme assay, survival assay)
- A method to keep the worms alive *in vitro* during incubation and downstream phenotypic analysis.
- A thorough assessment of whether the control PPMO (with no-target transcripts in the organism) and any carrier solvents have detrimental effects on the worms in the assay.

This final point is particularly pertinent to the use of PPMOs with scour worms as PPMOs require to be at relatively high concentrations in the culture medium (90µM in the case of the work published by Witola et al., (2016). Preliminary work with exsheathed *Telc*L3 has suggested that micromolar concentrations of a control and a gene-specific PPMO are both toxic to this stage of the worm (Nisbet et al. unpublished).

Table 6. Summary of gene silencing in *H. contortus, T. circumcincta* and *T. colubriformis*

Species	Stage	Target gene(s)	Evaluation	Reference
Hco	L3, L4, adult reduced L3 motility	β-tubulin	Reduced mRNA, reduced L4 development	Kotze et al. 2005
	L1-L3	β-tubulin GATA TF Hsp70 Cathepsin L ATPase Superoxide dismutase Paramyosin Collagen Intermediate filament COPII component Calcium binding protein	Reduced mRNA for some targets	Geldhof et al. 2006
	L3	H11 ASP-1 β-tubulin GTPcyclohydrolase Aquaporin *apq-2* Helicase *phi-10* Transcription factor *ceh-6* Chloride channel *exc-4* Ribosomal genes	Reduced mRNA for some targets Reduced in vivo survival H11 RNAi	Samarsinghe et al. 2011
	L1, L3, adult	Ubiquitin β-tubulin paramyosin Tropomyosin *vha-19* *noah-1* *mitr-1* *glf-1* *pat-12*	Reduced mRNA for some targets Reduced viability following RNAi feeding	Zawadzki et al. 2012
	Adult	*PMT1&2 Phosphoethanolamine N-methyltransferase	Reduced protein& reduced viability	Witola et al. 2016
Tci	L3	ASP	Reduced mRNA	Tzelos et al. 2015
Tco	L1	Tropomyosin Ubiquitin	Delayed development to L3	Issa et al. 2005

All studies carried out by RNAi except * which used morpholino oligomers

5.5 *In vitro* culture

Technology for improved *in vitro* culture of GI nematodes would provide a valuable system to deliver RNA or DNA for gene silencing or screen for effective neutralizing antibodies and establish vaccine screening platforms. Ideally, these would be comparable to current drug screening systems, where effects on worm motility, moulting or survival could be detected using automated systems. Whereas drug effects can often be monitored over a short time frame, gene and protein knockdown and subsequent effects on worm biology require a longer time in which the parasites must remain viable. Previous work reported *in vitro* development of *Hc* infective larvae to reproducing adult worms using abomasal extracts (Stringfellow, 1986; P. Presidente, unpublished). More recent studies have used cell co-culture to achieve improved development of GI nematode larvae. For example, co-culture with Caco2 cells, a human gut epithelial cell line previously used for *T.spiralis* culture (Gagliardo et al. 2002), promotes development of *Hc*L3 to L4 stage (Britton et al., 2016). Similarly, the human colonic cell line HT-29 supports the development of *Tc* L3 larvae (Andronicos et al., 2012). Optimisation of co-culture systems and/or supplementation with parasite growth factors may provide suitable systems for parasite development and longterm maintenance to identify essential genes and metabolic pathways.

An "*in vitro* direct challenge" (IVDC) method has been developed to examine the initial immune exclusion/prompt rejection mechanism operating in the abomasa of sheep rendered immune to *Telc* (Jackson et al., 2004) or *Hc* (Kemp et al., 2009) by continuous "trickle" infection. In this model, abomasal tissue explants were maintained in Hanks/Hepes solution (pH 7.6) in a high oxygen concentration, then challenged with exsheathed L3 nematode larvae. L3s that had successfully penetrated gastric glands or had been excluded/rejected were then recovered. While this technique is unlikely to be able to maintain parasitic stages of the nematodes for periods longer than a few hours, the methodology was adapted by Nisbet et al., (2009) to establish whether antibodies, raised against the *Telc* vaccine candidate surface-associated antigen-1 (Tci-SAA-1) and bound to the surface of exsheathed L3 *Telc*, were able to inhibit the larvae from establishing in abomasal explants from helminth-naïve lambs during short incubation periods, though no inhibition was observed.

Recent successful development of intestinal organoids (Sato et al., 2011; Powell & Behnke, 2017; Hamilton et al., 2018) has identified another *in vitro* technology that may support GI nematode development for target screening. Intestinal organoids (mini-guts) are derived from single stem cells isolated from the intestinal crypts. These $LGR5^{+}$ stem cells divide and differentiate *in vitro* under suitable conditions (with growth factors) to produce all the cells characteristic of gut epithelium, including goblet cells, enterocytes, Paneth cells, tuft cells and enteroendocrine cells. Organoids can be serially passaged long-term and may provide a more physiological epithelium culture system in which parasite larvae may be maintained and host-parasite interactions examined. In a novel approach, Maria Duque-Correa (WSI, UK) is investigating parasite survival and host-parasite interactions following injection of *Trichuris muris* L1 larvae into mouse intestinal organoids (https://www.nc3rs.org.uk/intestinal-organoids-replacement-strategy-unravel-early-host-intestinal-epithelia-interactions). GI nematode infective larvae are larger in size than *T. muris,* making their introduction into 3D organoid structures more difficult. However, once established, 3D organoids can also be cultured as a 2D epithelial layer, which would enable greater access and penetration of parasites. This potentially provides a physiological system to develop and maintain veterinary nematodes for vaccine candidate screening. Alternatively ILC2 cells involved in Th2

responses in gut (helminths) and lungs (Neil et al., 2010) may provide an additional means to screen for the developmental intricacies of HxP reactivity.

5.6 microRNAs and extracellular vesicles

In addition to RNAi-mediated gene silencing, nematodes express small regulatory microRNAs (miRNAs) that function to silence genes at the post-transcriptional level. In the free-living nematode *C. elegans*, some of these miRNAs are known to play essential roles in regulating development and responses to environmental stress (reviewed in Britton et al., 2014). Recent research has shown that some of the miRNAs present in *C. elegans* are conserved in veterinary GI nematodes, including *H. contortus* and *Telc* (Winter et al., 2012, Gu et al., 2017), and likely act to regulate developmental pathways and metabolism. miRNAs can regulate networks of genes to give an amplified effect. While miRNAs may themselves be targets for therapeutic intervention (Britton et al., 2014), identifying the pathways they regulate as parasites develop within the host has the potential to identify novel control targets (Marks et al., unpublished).

Further to regulating endogenous genes within the parasite, miRNAs are also released from GI nematodes, including *Telc*, *Hc* (Gu et al., 2017) and *Heligmosomoides polygyrus* (Buck et al. 2014). miRNAs have been sequenced from excretory-secretory (ES) products, both in the supernatant and in extracellular vesicles (EV) isolated from ES by differential ultracentrifugation (Buck et al., 2014; Gu et al., 2017). miRNAs have been identified from ES of parasites maintained *in vitro*, however parasite-derived miRNAs have also been identified in host plasma following filarial infections, suggesting that they are also released *in vivo* (Tritten et al., 2014). Importantly, secreted miRNAs present in EV have been shown to modulate host immune response genes, suggesting that they may function at the host-parasite interface to promote parasite survival and/or suppress immune-mediated damage (Buck et al., 2014). Recently, the proteome of EVs released from Telc has been characterised and includes a number of putative immunomodulatory proteins which are targeted by both IgA and IgG antibodies from Telc infected sheep (Tzelos et al., 2016). Targeting EV through vaccination may therefore neutralize immunoregulatory effects and enhance immunity. Indeed, recent vaccination studies in mice using 1.5-3ug EVs prepared from (protective) ES products of cultured adult *H. polygyrus* or *T. muris* nematodes and inoculated without adjuvant, reduced worm burden by around 50%, by inducing antibodies that promote uptake and processing by antigen presenting cells (APC) (Coakley et al., 2017; Shears et al., 2018). EVs from *T. muris* contained around 125 proteins, such that through their uptake by APC, EVs may provide a novel vaccine formulation (Shears et al., 2018).

6 Field integration and evaluation of vaccines

a) Integration into field control programs

Vaccines against sheep nematodes are envisaged as a component of an IPM approach, which would reduce the dependence on anthelmintic treatments, but would not obviate their use. Vaccination provides a strategic "epidemiological" effect through the suppression of pasture contamination with worm eggs and consequent reduction in worm burdens during the following months. As with present programs utilising anthelmintics, treatments are most efficient if given at times of the year when worm larval populations on pasture are relatively low, to prevent the development of significant worm burdens and subsequent larval challenge to sheep. Although vaccination is not an alternative to non-chemical approaches, the prolonged activity of vaccines would be beneficial where there are limited opportunities to use pasture management to avoid the grazing of worm-contaminated areas by worm-susceptible sheep.

Annual vaccination schedules will vary between locations, depending on environmentally-determined variations in the importance of different nematode species, and their seasonal patterns of worm challenge. Fortunately, ecological research over many years has provided a detailed understanding of seasonal patterns for the major species in the major "type" environments (O'Connor et al., 2006), which will serve as the basis for computer simulation modelling of optimal vaccination schedules for various locations (Dobson et al., 2011; Laurenson & Kahn 2018), followed by in-field validation.

However, although vaccination will reduce the requirement for anthelmintics, drenching will still be required in some instances, to remove worm species not covered by a vaccine, to treat significant infections in sheep not vaccinated, or where overwhelming challenge reduces the effectiveness of a vaccine. The obvious benefit of vaccines is in relation to anthelmintic resistance, to provide effective control where drenches are compromised by resistance, and to minimise the further development of resistance by decreasing the requirement for drenching. Further, it appears unlikely that resistance to vaccine antigens is likely to develop as has resistance to anthelmintics (D. Smith pers. com.). This would have the additional advantage that routine testing for efficacy would not be required, that the uncertainty of control effectiveness would not apply, and that the use of vaccines at strategic times when anthelmintics would be avoided (due to selection of drench resistance selection) could occur.

Long-acting anthelmintics could be considered a competitor to the use of vaccines, but they have significant disadvantages. Widespread resistance to the actives in these products (benzimidazoles and macrocyclic lactones) has significantly reduced their effectiveness on many sheep properties, especially against *Telc* and *Hc*. While they remain relatively effective against *Trichostrongylus* and the less-significant worm species, the heavy selection pressure for the development of resistance is well-recognised (Le Jambre et al., 1999), and is increasingly understood by users. Both vaccines and long-acting anthelmintics require more effort for administration (as injectables, or for the anthelmintics, oral capsule devices) than oral short-acting drenches, but the widespread use of the capsules indicates that many sheep farmers accept this requirement as a necessary trade-off for the longer-term effects.

b) Vaccine evaluation

Veterinary vaccines must be registered by the appropriate government authority before they can be sold. All must undergo and pass formal safety and efficacy tests in the final host, a slow and expensive process if the vaccine is to be registered for farm animal species like the sheep.

Registering a vaccine for one or more scour worm species with the Australian Pesticide and Veterinary Medicines Authority (APVMA) should be slightly easier than for Barbervax as it was the first vaccine to be registered for any parasitic nematode in Australia. Numerous questions arose, especially “how good does the vaccine have to be?”, but these issues were readily resolved often at face to face meetings at the APVMA in Canberra.

Given that a vaccine for *Hc* is already available commercially, it is suggested that initially at least, research on sheep worm vaccines should concentrate on *Telc* and *Tc* as they are the most economically important scour worm species for the 60% of animals in the winter rainfall zones and since considerable progress is being made with a recombinant vaccine for *Telc*

c) Experimental testing in penned worm free sheep

The work for the registration process should only start if preliminary trials have already indicated that a standard candidate vaccine formulation can repeatedly protect sheep against a standard challenge dose of worms. At least one adjuvant trial would be required at the outset so that a suitable formulation can be selected for all subsequent protection trials. For speed, simplicity and cost, it is probably better if a bolus challenge is used initially and then, once such trials are successful, a more realistic trickle challenge could be given.

The APVMA demand that three batches of vaccine at two dose rates are required for primary efficacy testing. The effect of maternal vaccine antibody either passively protecting the lamb or interfering with its response to vaccination must be evaluated. Both the onset and duration of protective immunity following vaccination need to be determined. Is vaccine immunity boosted by infection? How frequently would booster vaccinations need to be given? Is vaccination compatible with other treatments e.g. Clostridial or Barbervax vaccines? How stable is the vaccine? Can it be stored at room temperature or is it more stable at 4C or frozen?

The great majority of these questions can only be answered by vaccine challenge trials in sheep, making it an expensive process, especially as separate trials would have to be done for each of the worm species.

Efficacy would be judged by comparing the following parameters in vaccinates and controls: FWEC, body weight and or condition score, wool yield and quality and, for *Telc*, blood pepsinogen concentrations. These parameters could be measured fortnightly, but in a few trials it might also be valuable to kill some sub-groups at intervals to measure the kinetics of worm numbers as well as any differences in the degree of worm development with time.

d) Field evaluation

Only when it was reasonably clear that an effective candidate vaccine had been identified from the pen experiments, should field trials with it begin. These could start by vaccinating pregnant ewes

using the optimum regime determined by the pen trials. The ewes' offspring should also be immunised, again using the optimal regime identified by the pen trials. Serum anti- vaccine antibody responses would be monitored at regular intervals and protective immunity assessed as before by comparing FWEC (including speciation), body weight and or condition score, wool yield and quality and for *Telc*, blood pepsinogen concentrations.

Each candidate vaccine would have to be tested in field trials with lambs, hoggets and ewes. Such trials would have to be repeated in the temperate, summer and winter rainfall climatic zones typical of the main sheep producing areas of Australia.

About a dozen or so field trials would be needed.

In follow up and ongoing monitoring of efficacy for any development of "vaccine resistance", analysis of any genomic or proteomic changes in those worms and their progeny surviving vaccine-induced immunity would be needed as has been performed for survivors of Barbervax (Salle et al., 2018).

e) Commercialisation

Before any veterinary vaccine can be commercialised, it must be clear that an attractive market for such a product exists, that cost effective scale-up manufacture is possible and at a price competitive with existing control measures. Because substantial up-front investment is required for both field evaluation, registration and scale-up manufacture, commercialisation of veterinary vaccines discovered by academic institutions is usually done in partnership with a large pharmaceutical company. (This was not the route adopted for Barbervax.)

However, while vaccines are likely to be relatively costly compared to short-acting oral anthelmintics, the cost should be considered against the total worm-control cost. At present, the standard requirement for 5 injections of Barbervax retails at a similar cost to the most effective slow-release capsule (an abamectin-albendazole combination), but the series of injections provides a longer period of protection. Both Barbervax and the capsule also require at least one additional anthelmintic treatment. Marketed in the light of the benefits of the improved worm control from a vaccine compared to existing drench-based programs, and the absence of resistance problems that limit anthelmintic longevity, vaccines against scour worms are likely to prove economic and sustainable in the major sheep-raising regions of Australia. Adoption of a nematode vaccine would be enhanced if only 2-3 injections were required and these could be given when sheep are mustered for routine husbandry operations, as the larger number of Barbervax treatments needed for protection over the risk period is considered a limitation on uptake by farmers.

If it could be manufactured cost effectively, a bivalent vaccine highly protective for both *Telc* and *Tc,* would be commercially attractive, especially if combined with a *Hc* vaccine like Barbervax. The same would be unlikely for monovalent vaccines for some of the minor intestinal species e.g. *T. vitrinus, Nematodirus* or *Cooperia* and so it would seem unlikely that vaccination could ever completely replace anthelmintic treatment as a way of controlling sheep nematode parasites.

However, the removal of even one of the major scour worms as a major threat is likely to significantly reduce the overall impact of parasitism, and control of the remaining species is likely to be simplified and require less drenching (as experienced for *Trichostrongylus*, when *Hc* is controlled

using Barbervax). Should better worm control include a significant decrease in the severity of scouring, the consequent reduction in blowfly strike risk would provide a major additional incentive for vaccination.

As a target for vaccine development, at present *Telc* may be considered as the priority species. Resistance to anthelmintics is especially prevalent and severe in *Telc* (and *Hc*), as so far, the macrocyclic lactones (MLs) remain highly effective against *Trichostrongylus* and the minor worm species, with few verified reports of resistance on the basis of definitive investigations (Besier, 2013; Playford et al., 2014). As *Teladorsagia* is believed to comprise a single important species (i.e.,*Telc*; Grillo et al., 2008), the likelihood of between-species differences in vaccine targets may be lower in *Teladorsagia* compared with the multi-genus *Trichostrongylus*.

7 Recommendations for Stage 1 process of implementation

a) Building research teams and integrated expertise.

Many of the recommendations listed below are complex and require coordinated and integrated approaches *in vivo, ex vivo* and *in vitro*, together with substantial data processing capability (Figure 4). A successful vaccine against scour worms would occupy a 5-10 year horizon to develop, assess and implement into existing IPM schemes. This suggests a joint and collaborative approach, perhaps akin to the GINTIP/ SheepGenomics/ Parasol/ PARAVAC/ANTIdotE consortia involving domestic and international laboratories in the past and current. The vagaries of funding streams in the past decades have resulted in diminution, aging (retirement) and dissolution of cohesive expertise, such that one salutary requirement is a commitment to more longer-term, stable funding regimen directly supported through attainment of agreed milestones if career-based, capacity and capability is to be maintained or attractive for capable and innovative young researchers. It is also vital that parasitologists in this area have opportunities to interact with Industry and producers to both know and deal with parasite problems and the role for vaccines.

The second component for the investigations is to ensure a ready supply of quality-assured (QA) scour worm parasites for antigen/ DNA isolation and challenge. As capacity has contracted over the past 2 decades, fewer fresh, (verified pure) supplies are available and the infectivity of cryo-preserved isolates is unknown.

b) Short-term gains from low-hanging fruit.

There are several ongoing areas which have potential for success in the shorter term. These include;

- Some recent progress has been made with recombinant sub-unit proteins as vaccines for *Hc* (Hc23) and from *Telc* (8-antigen cocktail), as reviewed in the above sections. With the availability of low-cost sequencing technologies for both transcriptomic and genomic analyses, a cross-species bioinformatic analysis of the transcriptomes of all parasitic stages of the key species to search for homologues of these vaccine antigens could be rapid and relatively low cost. This would establish whether there are homologues of the antigens/predicted epitopes across species and, if so, that they are expressed in an appropriate stage of the parasite to target with a vaccine approach. Dependent on the outcome of this approach, a vaccine trial using either a single representative antigen across species or a concatenated antigen may be appropriate. The outputs would also inform the future rational selection of novel antigens using the technologies described in the "longer term strategic" approach described below.

- Vaccination with CarLA. The antigen is readily extracted from large number of L3 (supplied as above), but initial trials were suspended due to problems encountered with vaccine design and formulation. If the leads from the inhibition of establishment of *Telc* L3 (Harrison et al., 2008) can be verified *in vivo*, initial progress can be accomplished.

- Testing intranasal delivery *ala* hookworm and *Hc* (Fazwi et al., 2013; 2015) offers a delivery approach for consideration alongside rectal application or other approaches and formulations (McClure, 2009).

c) Longer term strategic inputs from teams

Investment in new technologies: while understanding mechanisms of immunity to scour worms is relevant to vaccine formulation, delivery and quantifying measures of protection, key to vaccine success is targeting the most appropriate antigens. We are currently lacking effective tools to identify which antigens will stimulate a response that has a detrimental impact on worm development, reproduction and/or survival. Investment in technologies including RNAi gene silencing and CRISPR/Cas9 knockout will provide the means to identify genes with essential function. Combined with bioinformatics data (genomic, transcriptomic, protein structural modelling, epitope prediction) vaccine development can focus on essential genes that are expressed in parasitic lifecycle stages, in accessible sites (e.g worm intestine, surface, excretory-secretory system) and are predicted to encode strong immunogenic epitopes. Furthermore, peptide array technologies are now at the stage that a significant proportion of the parasite proteome can be printed on a single chip which can be then used to unambiguously identify peptides targeted by antibodies from immune sheep. It is timely to establish these technologies and test proof of principle. This has the potential to provide a much-needed rational approach to vaccine design using the principles of reverse vaccinology, although this approach will still require moderate to large scale testing of antigen-pools in animal challenge models (e.g. Nkando et al., 2016; Tabor et al 2017: B.AHE.0212).

- **Induction of host immunity during the host-parasite interaction *in vivo.***
  Approaches to examine the kinetics of the host-parasite relationship have utilised classical techniques including sequential necropsies (McClure et al., 1991), surgical approaches such laparoscopy, indwelling fistulae and endoscopy (Rowe et al, 2009; Jones & Emery, 1994), supplemented by ELISAs and assays for host and parasite products such as antibodies, cytokines and mediators. Additional incisive power to determine the evolution of the parasitic life-cycles and host responses has been supplied by genomic, transcriptomic and proteomic technologies (eg McNeilly et al., 2017), using sequential samples. Both approaches are being utilised for the abomasal parasites (*Hc* and *Telc*) with current investigations. Similar approaches need to be developed and applied for intestinal GIN to define critical stages and pathways of the HxP interaction where vaccine formulations can intervene successfully (Figure 5).

  Consequently, comparable approaches are required to determine the effects of mucosal "adjuvants" delivered orally or applied to mucosal surfaces in attempts to match with protective responses developed by actual infection (Figure 4). In the absence of definitive information of the (stage-specific) protective responses or its induction in ruminants, most vaccines containing purified or recombinant antigens have been formulated and given by a "suck it and see" approach following methods described in the literature. A meta-analysis of the inductive capabilities of current vaccine adjuvants for ruminants may assist here.

- **Vaccine efficacy and effects on worm populations**:
  One of the key questions remains, "How much protection is sufficient for my vaccine?" Several models are poised to test hypotheses (Dobson et al., 2011; Laurenson et al., 2013; de Cistneros et al., 2014). Previous modelling studies (exemplified in Figure 4 from 1993) indicated that prevention of deaths in weaners with concomitant reductions in pasture L3 could be achieved with vaccines which gave 70-80% protection (reduced FWEC) in ca 80% of vaccinated animals responding. Death was presumed to occur with worm burdens of >2500. These simulations

should be extended to incorporate the various vaccine efficacies in concert with additional interventions normally associated with IPM – for example the interactions between vaccine efficacy and nutrition (or strategic anthelmintic use) to give adequate protection. This will need empirical data from pen and field studies in which vaccines of known efficacy are tested, even if their protective capacity is lower than the previously determined minimum thresholds. At this point, since current IPM utilise seasonal pasture L3 levels from field data that is more than 30 years old, no modelling on the effects of vaccination on pasture L3 has been done.

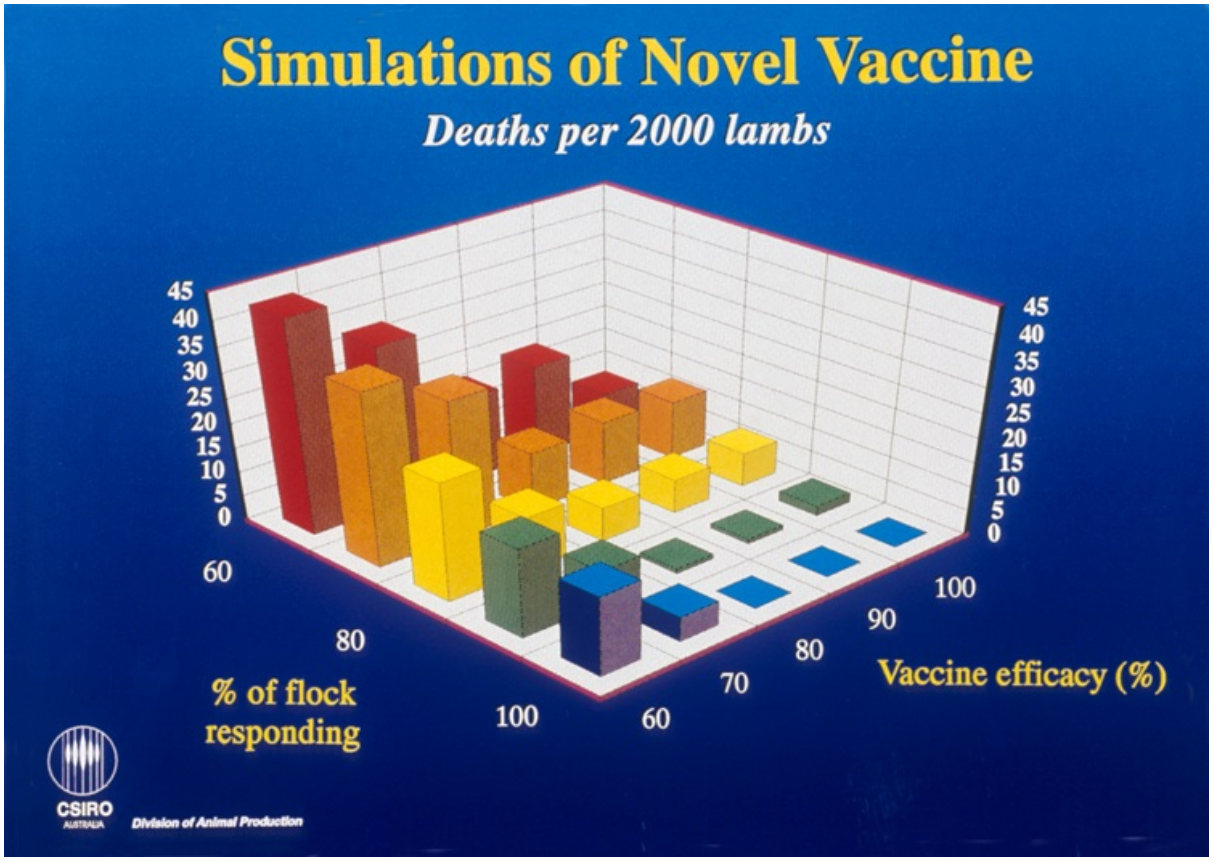

Fig. 4. Simulations of vaccines with varying levels of efficacy and differing proportions of host responders on the numbers of deaths from scour worms in a flock. Deaths occur in lambs when worm numbers reach 2500 (modified from Barnes et al., 1995 [by request in 1993]).

8 Bibliography

Abraham WM. 2008. Modeling of asthma, COPD and cystic fibrosis in sheep. Pulm. Pharmacol. Ther. 21:743–54.

Abraham, WM., J. C. Delehunt, L. Yerger, and B. Marchette. 1983. Characterization of a late phase pulmonary response following antigen challenge in allergic sheep. Am. Rev. Respir. Dis. 128:839–844.

Adams, D.B., 1993. Systemic responses to challenge infection with *Haemonchus contortus* in immune Merino sheep. Vet. Res. Comm. 17, 25-35.

Adams, DB., Colitz, IG. 1991. Immunity to *Haemonchus contortus* and the cellular response to helminth antigens in the mammary gland of non-lactating sheep Int. J. Parasitol. 21: 631-639.

Albers GAA, Gray GD, Piper LR, Barker JSF, Lejambre L, Barger IA. The genetics of resistance and resilience to *Haemonchus contortus* infection in young Merino sheep. Int J Parasitol. 1987;17:1355-63.

Anderson N. Trichostrongylid infections of sheep in a winter rainfall region. I. Epizootiological studies in the Western District of Victoria, 1966–1967. Aust. J. Agric. Res. 1972; 23;1113-1129.

Anderson N. Trichostrongylid infections of sheep in a winter rainfall region. II. Epizootiological studies in the Western District of Victoria, 1967–1966. Aust. J. Agric. Res. 1973; 24:599-611.

Anderson NA, Morris RS, McTaggart IK. An economic analysis of two schemes for the control of helminthiasis in weaned lambs. Aust. Vet. J. 1976; 52:174-180.

Anderson N, Dash KM, Donald AD, Southcott WH, Waller PJ. Epidemiology and control of nematode infections. In “Theepidemiology and control of gastro-intestinal parasites of sheep in Australia” (Eds. Donald AD, Southcott WH, Dineen JK). CSIRO Publications, Australia. 1978 pp 23-51.

Andronicos, N., Hunt, P., Windon, R., 2010. Expression of genes in gastrointestinal and lymphatic tissues during parasite infection in sheep genetically resistant or susceptible to *Trichostrongylus colubriformis* and *Haemonchus contortus.* Int. J. Parasitol. 40, 417–429.

Andronicos NM, McNally J, Kotze AC, Hunt PW, Ingham A. 2012. *Trichostrongylus colubriformis* larvae induce necrosis and release of IL33 from intestinal epithelial cells in vitro: Implications for gastrointestinal nematode vaccine design. Int. J. Parasitol. 42:295–304.

Angulo-Valadez CE, Ascencio F, Jacquiet P, Dorchies P, Cepeda-Palacios R. 2011. Sheep and goat immune responses to nose bot infestation: a review. Med. Vet. Entomol. 25:117–25.

Asojo OA, Darwiche R, Gebremedhin S, Smant G, Lozano-Torres JL, Drurey C, Pollet J, Maizels RM, Schneiter R, Wilbers RHP.2018. *Heligmosomoides polygyrus* venom allergen-like protein-4 (HpVAL-4) is a sterol binding protein. Int J Parasitol. 48:359-369.

Assenza F, Elsen JM, Legarra A, Carre C, Salle G, Robert-Granie C, et al.2014. Genetic parameters for growth and faecal worm egg count following Haemonchus contortus experimental infestations using pedigree and molecular information. Genet. Sel. Evol. 46:13.

Astronomo RD, Burton DR. 2010. Carbohydrate vaccines: developing sweet solutions to sticky situations? Nat Rev Drug Discov. 9:308-324.

Bahirathan M, Miller JE, Barras SR, Kearney MT. Variation in susceptibility of Suffolk and Gulf Coast Native suckling lambs to naturally acquired strongylate nematode infections. Vet Parasitol. 2000;65:259-68.

Bailey JN, Kahn LP, Walden-Brown SW. The relative contributions of *T. colubriformis, T. vitrinus, T. axei and T. rugatus* to sheep infected with *Trichostrongylus* spp. on the northern tablelands of New South Wales. Vet. Parasitol. 2009; 65:88-95.

Bailey JN, Kahn LP, Walden-Brown SW. Comparison of strategies to provide lambing paddocks of low gastro-intestinal nematode infectivity in a summer rainfall region of Australia. Vet. Parasitol. 2009; 161:218-231.

Baker RL, Mwamachi DM, Audho JO, Aduda EO, Thorpe W. Genetic resistance to gastro-intestinal nematode parasites in Red Maasai, Dorper and Red Maasai x Dorper ewes in the sub-humid tropics. Anim Sci. 1999;69:335-44.

Baker RH, Britton C, Roberts B, Loer CM, Matthews JB, Nisbet AJ. 2012. Melanisation of *Teladorsagia circumcincta* larvae exposed to sunlight: a role for GTP-cyclohydrolase in nematode survival. Int J Parasitol. 42:887-891.

Bakker, N., Vervelde, L., Kanobana, K., Knox, DP., Cornelissen, A.W., de Vries, E., Yatsuda, AP., 2004. Vaccination against the nematode Haemonchus contortus with a thiol-binding fraction from the excretory/secretory products (ES). Vaccine 22, 618–628.

Bain, RK. 1999. Irradiated vaccines for helminth control in livestock. Int. J. Parasitol. 29:185-191.

Barnes, E.H., Dobson, R.J, Barger, I.A. 1995. Worm control and anthelmintic resistance; adventures with a model. Parasitol Today. 11:56-63

Barger, I.A. 1988. Resistance of young lambs to *Haemonchus contortus* infection, and its loss following anthelmintic treatment. Int. J. Parasitol. 18: 1107–1109.

Barger, I. A., Le Jambre, L.F., Georgi, J. R., Davies, H.I., 1985. Regulation of *Haemonchus contortus* populations in sheep exposed to continuous infection. Int. J. Parasitol. 15, 529-533.

Barger IA. The role of epidemiological knowledge and grazing management for helminth control in small ruminants. Int. J. Parasitol. 1999; 29:41-47

Barker IK. Location and distribution of T*richostrongylus colubriformis* in the small intestine of sheep during the prepatent period, and the development of villus atrophy. J. Comp. Pathology 1975; 85:417-426.

Barker, IK., Titchen, DA. 1982. Gastric dysfunction in sheep infected with, a nematode inhabiting the small intestine. Int. J. Parasitol., 12: 345-356.

Barnes EH, Dobson RJ, Barger IA. Worm control and anthelmintic resistance: adventures with a model. *Parasitol. Today* 1995**;** 11:56-63.

Beasley AM, Kahn LP, Windon RG. The periparturient relaxation of immunity in Merino ewes infected with *Trichostrongylus colubriformis:* Parasitological and immunological responses. Vet. Parasitol. 2010; 168:60-70.

Beh, K.J., Lascelles, A.K.1981. The effect of route of administration of antigen on the antibody-containing cell response in lymph of sheep. Immunology 41: 577- 582.

Bendixsen, T., Emery, D.L. Jones, W.O. 1995. The sensitization of mucosal mast cells during infections with *Trichostrongylus colubriformis* or *Haemonchus contortus* in sheep. Int. J. Parasitol. 25, 741-748

Bendixsen, T., Windon, RG., Huntley, JF., McKellar, A., Emery, DL. 2004. Development of a new monoclonal antibody to ovine chimeric IgE and its detection of systemic and local IgE antibody responses to the intestinal nematode *Trichostrongylus colubriformis.* Vet. Immunol. Immunopath. 97: 11-24.

Benavides MV, Sonstegard TS, Van Tassell C. Genomic Regions Associated with Sheep Resistance to Gastrointestinal Nematodes. *Trends Parasitol.* 2016;32(6):470-480.

Besier RB. Management of helminth parasites of sheep in Australia. (2004) Proceedings 355: Sheep Medicine (Course May 2004) (Post graduate Foundation in Vet. Science, University of Sydney) Chapter 10, pp 129-172.

Besier RB. Sheep disease control plans, internal parasites: Wormkill, Drenchplan, Wormplan, Wormbuster, Wormcheck, Wormwise. Proc. 4th Int. Sheep Vet. Congress, Armidale, NSW, 1997; pp 74 – 80.

Besier RB. Refugia-based strategies for sustainable worm control: factors affecting the acceptability to sheep and goat owners. Vet. Parasitol. 2012; 186: 2-9.

Besier RB, Lyon J. Summer drenching of sheep: new recommendations for high rainfall areas. *West Aust. J. Agric.* 1990; 31: 103-107.

Besier RB, Love SCJ. Anthelmintic resistance in sheep nematodes in Australia: the need for new approaches. Aust. J. Exp. Agric. 2003; 43:1383-1391.

Besier, R.B., Khan, L.W., Sargison, N.D., Van Wyk, J.A ., 2016. The pathophysiology, ecology and epidemiology of *Haemonchus contortus* infection in Small Ruminants. Adv. Parasitol. 93, 95-143.

Besier, R.B., Love, R.A., Lyon, J., van Burgel, A.J., 2010. A targeted selective treatment approach for effective and sustainable sheep worm management: investigations in Western Australia. Anim. Prod. Sci. 50, 1034–1042.

Besier RB, Kahn LP. Paraboss – the national sheep parasite coordination program. Proc. Ann. Conf. Aust. Sheep Veterinarians. 2015; pp 62-65.

Besier B, Kahn L, Dobson R, Smith D. Barbervax – a new strategy for *Haemonchus* management. Proc. Ann. Conf. Aust. Sheep Veterinarians 2015; pp 373-377.

Beveridge I, Pullman AL, Martin RR, Barelds, A.1989a. Effects of temperature and relative humidity on development and survival of the free-living stages of *Trichostrongylus colubriformis*, *T. rugatus* and *T. vitrinus.* Vet. Parasitol., 33: 143-153.

Beveridge I, Pullman AL, Phillips PH, Martin RR, Barelds A, Grimson R. 1989b.Comparison of the effects of infection with *Trichostrongylus colubriformis, T. vitrinus and T. rugatus* in Merino lambs. Vet. Parasitol. 32:229-245

Bischof, RJ., Snibson, K., Shaw, R., Meeusen, ENT. 2003. Induction of allergic inflammation in the lungs of sensitized sheep after local challenge with house dust mite. Clin Exp Allergy 33:367-375

Bishop S, Bairden K, McKellar Q, Park M, Stear M. Genetic parameters for faecal egg count following mixed, natural, predominantly *Ostertagia circumcincta* infection and relationships with live weight in young lambs. Anim Sci. 1996;63(03):423-8.

Bisset S.A., Morris, C.A., Squire, D.R., Hickey, S.M. 1996. Genetics of resilience to nematode parasites in young Romney sheep - use of weight gain under challenge to assess individual treatment requirements. New Zealand J. Agric. Res. 39, 313-323.

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

Blitz, N. M., Gibbs, H. C., 1972. Studies on the arrested development of *Haemonchus contortus* in sheep—I. The induction of arrested development. Int. J. Parasitol. 2, 5–12.

Bouix J, Krupinski J, Rzepecki R, Nowosad B, Skrzyzala I, Roborzynski M, et al. Genetic resistance to gastrointestinal nematode parasites in Polish long-wool sheep. Int J Parasitol. 1998;28:1797-804.

Britton C, Marks ND, Roberts AB.2016. Functional genomics tools for *Haemonchus contortus* and lessons from other helminths. Adv Parasitol 93: 599-623.

Britton C, Murray L. 2006. Using *Caenorhabditis elegans* for functional analysis of genes of parasitic nematodes. International Journal for Parasitology 36:651–659.

Britton C, Winter AD, Gillan V, Devaney E. 2014 MicroRNAs of parasitic helminths – Identification, characterization and potential as drug targets. International Journal for Parasitology: Drugs and Drug Resistance 4:85–94.

Brown, WC., Rice-Ficht. A., Estes, M. 1998. Bovine type 1 and type 2 responses. Veterinary Immunology and Immunopathology, 63: 45-55.

Brunsdon RV. Principles of helminth control. Parasitol. 1980; 6:185-215.

Buck, A.H., Coakley, G., Simbari, F., McSorley, H.J., Quintana, J.F., Le Bihan, T., Kumar, S., Abreu-Goodger, C., Lear, M., Harcus, Y., et al. 2014. Exosomes secreted by nematode parasites transfer small RNAs to mammalian cells and modulate innate immunity. Nat. Commun. 5: 5488.

Callinan A.P.L. 1978. The ecology of the free-living stages of *Trichostrongylus axei.* Int. J. Parasitol. 8: 453–456.

Callinan APL. The ecology of the free-living stages of *Trichostrongylus vitrinus*. Int. J. Parasaitol. 1979; 9:133-136.

Callinan APL. The ecology of the free-living stages of *Ostertagia circumcincta*. Int. J. Parasaitol. 1978; 8:233-237.

Cantacessi C, Mitreva M, Campbell BE, Hall RS, Young ND, Jex AR, Ranganathan S, Gasser RB. 2010. First transcriptomic analysis of the economically important parasitic nematode,

Trichostrongylus colubriformis, using a next-generation sequencing approach. Infection, Genetics and Evolution 10; 1199–1207.

Chen, C., Fenk, L.A., de Bono, M. 2013. Efficient genome editing in *Caenorhabditis elegans* by CRISPR-targeted homologous recombination. Nucleic Acids Res. 41:e193.

Coakley G, McCaskill JL, Borger JG, Simbari F, Robertson E, Millar M, Harcus Y, McSorley HJ, Maizels RM, Buck AH. 2017. Extracellular vesicles from a helminth parasite suppress macrophage activation and constitute an effective vaccine for protective immunity. Cell Rep 19:1545-1557.

Coop RL, Kyriazakis I.1999. Nutrition-parasite interaction. Vet Parasitol.84:187-204.

Coop, R. L., Kyriazakis, I. 2001. Influence of host nutrition on the development and consequences of nematode parasitism in ruminants. Trends in Parasitology 17: 325-330.

Coop, R. L., Huntley, J. F., Smith, W.D., 1995. Effect of dietary protein supplementation on the development of immunity to *Ostertagia circumcincta* in growing lambs. Res. Vet. Sci., 59: 24-29.

Cornelius MC, Jacobson C, Dobson RJ, Besier RB. Computer modelling of anthelmintic resistance and worm control outcomes for refugia-based nematode control strategies in Merino ewes in Western Australia. Vet Parasitol. 2016; 220: 59-66.

Coulter A, Harris R, Davis R, Drane D, Cox J, et al. (2003) Intranasal vaccination with ISCOMATRIX adjuvanted influenza vaccine. Vaccine 21: 946-949.

Darwiche R, Lugo F, Drurey C, Varossieau K, Smant G, et al. 2018. Crystal structure of *Brugia malayi* venom allergen-like proten-1 (Bm-VAL-1), a vaccine candidate for lymphatic filariasis. Int. J Parasitology 48:371-378.

Daynes RA, Enioutina EY, Butler S, Mu HH, McGee ZA, et al. (1996) Induction of common mucosal immunity by hormonally immunomodulated peripheral immunization. Infect Immun 64: 1100-1109.

de Chaneet GC, Dunsmore JD.Climate and the distribution of intestinal Trichostrongylus spp. of Sheep. Vet. Parasitol. 1988; 26:273-283.

de Cisneros P.J, Stear MJ, Mair C, et al. 2014.An explicit immunogenetic model of gastrointestinal nematode infection in sheep. Journal of the Royal Society, Interface / the Royal Society. 11; 99.

de Souza C, Lopes MD, De Oliveira FM, Passos MJF, Ferreira LCG, Faria BF, Villar JAFP, Junior MC, Taranto AG, Dos Santos LL, Fonseca CT, de Oliveira Lopes D. 2018. Rational selection of immunodominant and preserved epitope Sm043300e from *Schistosoma mansoni* and design of a chimeric molecule for biotechnological purposes. Mol Immunol. 93:133-143.

Dea-Ayuela MA, Rama-Iniguez S, Torrado-Santiago S, Bolas-Fernandez F (2006) Microcapsules formulated in the enteric coating copolymer Eudragit L100 as delivery systems for oral vaccination against infections by gastrointestinal nematode parasites. J Drug Target 14: 567-575.

Dhar, D., & Singh, K. 1970. Effect of Irradiation on the Infective Stage Larvae of the Nematode, *Oesophagostomum columbianum* and their use as a vaccine. J. Helminthol. 44: 11-26. doi:10.1017/S0022149X00021404.

Dineen, K.K., Gregg, P. & Lascelles, A.K. 1978. The response of lambs to vaccination at weaning with irradiated *Trichostrongylus colubriformis* larvae: segregation into responders and nonresponders. Int. J. Parasitol. 8, 59-63.

Dineen J.K., Gregg, P., Windon, R.G.,Donald, A.D. and Kelly, J.D. 1977. The role of immunologically specific and non- specific components of resistance in cross-protection of intestinal nematodes. Int. J. Parasitol. 7, 211-215.

Dobrut A, Brzozowska E, Górska S, Pyclik M, Gamian A, Bulanda M, Majewska E, Brzychczy-Włoch M. 2018. Epitopes of Immunoreactive Proteins of Streptococcus Agalactiae: Enolase, Inosine 5'-Monophosphate Dehydrogenase and Molecular Chaperone GroEL. Front Cell Infect Microbiol. 8:349. doi: 10.3389/fcimb.2018.00349

Dobson, R.J., Barnes, E. H. 1995. Interaction between *Ostertagia circumcincta* and *Haemonchus contortus* infection in young lambs. Int. J. Parasitol. 25, 495-501.

Dobson, R.J., Barnes, E.H., Tyrrell, K.L., Hosking, B.C., Larsen, J.W.A., Besier, R.B.,Love, S., Rolfe, P.F., Bailey, J.N. 2011. A multi-species model to access the effect of refugia on worm control and anthelmintic resistance in sheep grazing systems. Aust. Vet. J. 89, 200–208.

Dobson, R.J., Waller, P.J., Donald, A.D. 1990. Population dynamics of *Trichostrongylus colubriformis* in sheep: The effect of infection rate on the establishment of infective larvae and parasite fecundity. Int. J. Parasitol. 20, 347-352.

Donald AD, Morley FHW, Waller PJ, Axelson A, Donnelly JR. Availability to grazing sheep of gastrointestinal nematode infection arising from summer contamination of pastures. Aust J. Agric. Res. 1978; 29:189-204.

Donald, A.D., Southcott, WH, Dineen, J.K. 1978: The Epidemiology and Control of Gastrointestinal Parasites of Sheep in Australia. Commonwealth Scientific and Industrial Research Organisation: Melbourne).

Donaldson, J., van Houtert, M.F.J., Sykes, A.R., 1998. The effect of nutrition on the periparturient parasite status of mature ewes. Anim. Sci. 67, 523-533.

Douvres, FW. 1980. *In vitro* development of *Trichostrongylus colubriformis*, from infective larvae to young adults. Parasitol. 66: 466-71.

Douvres, FW. 1983. The *in vitro* cultivation of *Oesophagostomum radiutum*, the nodular worm of cattle. iii.Effects of bovine heme on development to adults. J. Parasitol. 69: 570-576.

Doyle SR, Laing R, Bartley, DJ, Britton C, Chaudry U et al. 2017. A genome resequencing-based genetic map reveals the recombination landscape of an outbred parasitic nematode in the presence of polyploidy and polyandry. Genome Biology and Evolution 10:396-409.

East, I.J., Berrie, D.A. and Fitzgerald, C.J., 1988. *Oesophagostomum radiatum:* successful vaccination of calves with an extract of *in vitro* cultured larvae. Int. J. Parasitol., 18: 125-127.

East, l.J., Fitzgerald, C.J. and Bernie, D.A., 1993. *Oesophagostomum radiatum; the* effect of different adjuvants on vaccination with a partially purified protective antigen. Vet. Parasitol., 49: 191-200.

EIdridge, J.H., Staas, J.K., Meulbroek, J.A., McGhee, J.R., Tice, T.R. and Gilley, R.M., 1991. Biodegradable microspheres as a vaccine delivery system. Mol. lmmunol., 28: 287-294.

Ellis SE, Newlands GF, Nisbet AJ, Matthews JB. 2012. Phage-display library biopanning as a novel approach to identifying nematode vaccine antigens. Parasite Immunol. 34:285-95. doi: 10.1111/j.1365-3024.2011.01317.x.

Ellis SE. Interactions of *Teladorsagia circumcincta* with the ovine immune system – mimicry and vaccine development. PhD Thesis, University of Edinburgh, 2014.

Ellis S, Matthews JB, Shaw DJ, Paterson S, McWilliam HE, Inglis NF, Nisbet AJ. 2014. Ovine IgA-reactive proteins from *Teladorsagia circumcincta* infective larvae. Int. J Parasitol. 44:743-50. doi: 10.1016/j.ijpara.2014.05.007.

Emery, D.L., 1996. Vaccination against worm parasites of livestock. Vet. Parasitol. 64, 31-45.

Emery, DL., Beveridge IM 2015. "Australasian animal Parasites: Inside and Out". Australian Society for Parasitology. http://parasite.org.au/wp-content/assets/Parasitology2015.pdf.

Emery, D., Hunt, P., Le Jambre, L. 2016. *Haemonchus contortus*: the then and now, and where to from here? Int. J. Parasitol. 46, 755-769.

Emery, D.L., McClure, S.J. 1995. Studies on the inhibition of rejection of *Trichostrongylus colubriformis* larvae from immune sheep. Int. J. Parasitol. 25, 761-764.

Emery, D.L., McClure, S.J., Davey, R.J., Bendixsen, T. 1999. Induction of protective immunity to *Trichostrongylus colubriformis* in neonatal Merino lambs. Int. J. Parasitol. 29, 1037-1046.

Emery, DL., Rothel, JR., Wood, PR. 1990. The influence of antigens and adjuvants on the production of gamma-interferon and antibody by ovine lymphocytes. Immunology and Cell Biology 68:127-36.

Emery, D.L., Wagland, B.M., McClure, S.J., 1993. Rejection of heterologous nematodes by sheep immunized with larval or adult *Trichostrongylus colubriformis*. Int. J. Parasitol. 23, 841 846.

Enioutina EY, Bareyan D, Daynes RA (2008) TLR ligands that stimulate the metabolism of vitamin D3 in activated murine dendritic cells can function as effective mucosal adjuvants to subcutaneously administered vaccines. Vaccine 26: 601-613.

Fawzi EH., Bustos, TC., Samblas, MG., González-González, G., Solano, J., González-Sánchez, M-E. et al. 2013. Intranasal Immunization of Lambs with Serine/Threonine Phosphatase 2A against Gastrointestinal Nematodes. Clin. Vaccine Immunol. 20: 1352–1359.

Fawzi, E.M., González-Sánchez, M.E., Corral, M.J., Cuquerella, M. and Alunda, J.M. 2014. Vaccination of lambs against *Haemonchus contortus* infection with a somatic protein (Hc23) from adult helminths. Int. J. Parasitol. 44, 429-436.

Fawzi, E.M., González-Sánchez, M.E., Corral, M.J., Alunda, J.M. and Cuquerella, M. 2015. Vaccination of lambs with the recombinant protein rHc23 elicits significant protection against *Haemonchus contortus* challenge. Vet. Parasitol. 211, 54-59.

Félix MA. 2008. RNA interference in nematodes and the chance that favored Sydney Brenner. J Biol. 7: 34.

Fleischmann RD, Adams MD, White O, Clayton RA, Kirkness EF, Kerlavage AR, Bult CJ, Tomb JF, Dougherty BA, Merrick JM, et al. 1995. Whole-genome random sequencing and assembly of *Haemophilus influenzae* Rd. Science. 269:496-512.

Francis J.1996. Resistance of Zebu and other Cattle to Tick Infestation and Babesiosis with Special Reference to Australia: An Historical Review. Br Vet J.;122:301-7.

Frenkel MJ, Dopheide TA, Wagland BM, Ward CW. 1992. The isolation, characterization and cloning of a globin-like, host-protective antigen from the excretory-secretory products of *Trichostrongylus colubriformis*. Mol. Biochem. Parasitol. 50:27-36.

Gagliardo, L.F., McVay, C.S., Appleton, J.A., 2002. Molting, ecdysis, and reproduction of *Trichinella spiralis* are supported *in vitro* by intestinal epithelial cells. Infect. Immun. 2002;70: 1853-1859.

Gallichan WS, Woolstencroft RN, Guarasci T, McCluskie MJ, Davis HL, et al. (2001) Intranasal immunization with CpG oligodeoxynucleotides as an adjuvant dramatically increases IgA and protection against herpes simplex virus-2 in the genital tract. J Immunol 166: 3451-3457.

Gamble HR, Zajac AM. Resistance of St. Croix lambs to *Haemonchus contortus* in experimentally and naturally acquired infections. Vet Parasitol. 1992;41:211-25.

Gang SS, Castelletto ML, Bryant AS, Yang E, Mancuso N, Lopez JB, et al. 2017. Targeted mutagenesis in a human-parasitic nematode. PLoS Pathog 13: e1006675

Garnier, R., Grenfell, BT., Nisbet, AJ., Matthews, JB., Graham, AL. 2016. Integrating immune mechanisms to model nematode worm burden: an example in sheep. Parasitology 143: 894–904.

Geldhof, P., Visser, A., Clark, D., Saunders, G., Britton, C., Gilleard, J., Berriman, M. and Knox, D. P. 2007. RNA interference in parasitic helminthes: current situation, potential pitfalls and future prospects. Parasitology 134:609–619.

Geldhof, P., Murray, L., Couthier, A., Gilleard, J.S., McLauchlan, G., Knox, D.P., Britton, C. 2006. Testing the efficacy of RNA interference in *Haemonchus contortus*. Int. J. Parasitol. 36: 801–810.

Geldhof et al 2004 Parasite Immunol. 26(1):37-43.

Gerbe F, Sidot E, Smyth DJ, Ohmoto M, Matsumoto I, Dardalhon V, Cesses P, Garnier L, Pouzolles M, Brulin B, Bruschi M, Harcus Y, Zimmermann VS, Taylor N, Maizels RM, Jay P. 2016. Intestinal epithelial tuft cells initiate type 2 mucosal immunity to helminth parasites. Nature 529:226-230.

Gershwin, LJ. 2015. Comparative Immunology of Allergic Responses. Ann. Rev. Anim. Biosci.3:327-346

Gibbs HC. Hypobiosis in Parasitic Nematodes—An Update. Vet.Parasitol, 1986; 25:129-174.

Gibson, T.E., 1973. Recent advances in the epidemiology and control of parasitic gastroenteritis in sheep. Vet. Rec. 92, 469-473.

Gilleard JS, Redman E. 2016. Genetic diversity and population structure of *Haemonchus contortus.* Adv Parasitol. 93:31- 68.

González JF, Hernández -Á, Meeusen ENT, et al. 2011. Fecundity in adult Haemonchus contortus parasites is correlated with abomasal tissue eosinophils and gd T cells in resistant Canaria Hair Breed sheep. *Vet Parasitol.* 178:286-292.

Gonzales-Hernandez et al 2016. Host protective ASP-based vaccine against the parasitic nematode *Ostertagia ostertagi* triggers NK cell activation and mixed IgG1-IgG2 response. Scientific Reports 6: 29496.

González-Sánchez ME, Cuquerella M, Alunda JM. 2018. Vaccination of lambs against *Haemonchus contortus* with the recombinant rHc23. Effect of adjuvant and antigen dose. PLoS One. 13:e0193118.

Good B, Hanrahan JP, Crowley BA, Mulcahy G. Texel sheep are more resistant to natural, nematode challenge than Suffolk sheep based on faecal egg count and worm burden. Vet Parasitol. 2006;136:317-27.

Gossner, A. G., Venturina, V. M., Shaw, D. J., Pemberton, J. M. and Hopkins, J. 2012. Relationship between susceptibility of Blackface sheep to *Teladorsagia circumcincta* infection and an inflammatory mucosal T cell response. Veterinary Research 43, 26.

Greer AW, Huntley JF, Mackellar A, McAnulty RW, Jay NP, Green RS, Stankiewicz M, Sykes AR. The effect of corticosteroid treatment on local immune responses, intake and performance in lambs infected with *Teladorsagia circumcincta*. Int. J. Parasitol. 2008; 38:1717-1728.

Grillo V, Craig BH, Wimmer B, Gilleard JS. Microsatellite genotyping supports the hypothesis that *Teladorsagia davtiani* and *Teladorsagia trifurcata* are morphotypes of *Teladorsagia circumcincta. Mol Biochem Parasitol.* 2008;159(1):59-63.

Gutierrez-Gil B, Perez J, de la Fuente LF, Meana A, Martinez-Valladares M, San Primitivo F, et al. Genetic parameters for resistance to trichostrongylid infection in dairy sheep. Animal. 2010;4:505-12

Hagen, J., Young, N.D., Every, A.L., Pagel, C.N., Schnoeller, C., Scheerlinck, J-P.Y., Gasser, R.B., Kalinna, B.H. 2014. Omega-1 knockdown in *Schistosoma mansoni* eggs by lentivirus transduction reduces granuloma size in vivo. Nat. Commun. 5:5375.

Halliday AM, Lainson FA, Yaga R, Inglis NF, Bridgett S, Nath M, Knox DP. 2012. Transcriptional changes in *Teladorsagia circumcincta* upon encountering host tissue of differing immune status. Parasitology. 139:387-405. doi: 10.1017/S0031182011002010.

Hamilton CA, Young R, Jayaraman S, Sehgal A, Paxton E et al. 2018. Development of *in vitro* enteroids derived from bovine small intestinal crypts. Veterinary Research 49:54

Hansen CS, Østerbye T, Marcatili P, Lund O, Buus S, Nielsen M. 2017. ArrayPitope: Automated Analysis of Amino Acid Substitutions for Peptide Microarray-Based Antibody Epitope Mapping. PLoS One. 12:e0168453.

Harrison, G. B. L., Pulford, H. D., Gatehouse, T. K., Shaw, R. J., Pfeffer, A., Shoemaker, C. B. 1999. Studies on the role of mucus and mucosal hypersensitivity reactions during rejection of *Trichostrongylus colubriformis* from the intestine of immune sheep using an experimental challenge model. Int. J. Parasitol. 29: 459-468.

Harrison, G.B.L., Pulford, H.D., Doolin, E.E., Pernthaner, A., Shoemaker, C.B., Hein, W.R., 2008. Antibodies to surface epitopes of the carbohydrate larval antigen CarLA are associated with passive protection in strongylid nematode challenge infections. Parasite Immunol. 30, 577–584.

Hassan, M., Good, B., Hanrahan, JP., et al. 2011. The dynamic influence of the DRB1*1101 allele on the resistance of sheep to experimental *Teladorsagia circumcincta* infection Vet Res. 42: 46-55.

He Y, Xiang Z, Mobley HLT. 2010. Vaxign: the first web-based vaccine design program for reverse vaccinology and an

application for vaccine development. .*J Biomed Biotechnol*. 2010:297505.

Healey K, Lawlor C, Knox MR, Chambers M, Lamb J. Field evaluation of *Duddingtonia flagrans* IAH 1297 for the reduction of worm burden in grazing animals: Tracer studies in sheep. Vet. Parasitol. 2018; 253: 48-54.

Hein, W.R., Pernthaner, A., Piedrafita, D., Meeusen, E.N., 2010. Immune mechanisms of resistance to gastrointestinal nematode infections in sheep. Parasite Immunol. 32,541–548.

Herlich, H., Douvres, F., & Romanowski, R. (1973). Vaccination against *Oesophagostomum radiatum* by Injecting Killed Worm Extracts and *In-vitro*-Grown Larvae into Cattle. *The Journal of Parasitology, 59*(6), 987-993. doi:10.2307/3278631

Holmes, P. and Sackett, D. 1990. Weaner Series - target weights and feeding. Holmes & Sackett “On Farm” 3, 23-25.

Hoste H, Jackson F, Athanasiadou S, Thamsborg SM, Hoskin SO. The effects of tannin-rich plants on parasitic nematodes in ruminants. Trends Parasitol. 2006; 22:253–261.

Houdijk, J.G., 2008. Influence of periparturient nutritional demand on resistance to parasites in livestock. Parasite Immunol. 30, 113-121.

Houdijk, J.G., Kyriazakis, I., Coop, R.L., Jackson, F., 2001. The expression of immunity to Teladorsagia circumcincta in ewes and its relationship to protein nutrition depend on body protein reserves. Parasitology 122, 661-672.

Houdijk, J.G., Kyriazakis, I., Jackson, F., Huntley, J.F., Coop, R.L. 2003. Is the allocation of metabolisable protein prioritised to milk production rather than to immune functions in Teladorsagia circumcincta-infected lactating ewes? Int. J. Parasitol. 33, 327-338.

Houdijk, J.G., Jackson, F., Coop, R.L., Kyriazakis, I., 2006. Rapid improvement of immunity to Teladorsagia circumcincta is achieved through a reduction in the demand for protein in lactating ewes. Int. J. Parasitol. 36, 219-227.

Howitt MR, Lavoie S, Michaud M, Blum AM, Tran SV, Weinstock JV, Gallini CA, et al. 2016. Tuft cells, taste-chemosensory cells, orchestrate parasite type 2 immunity in the gut. Science 351:1329-1333.

Hu, Y, Ellis, BL., Yiu, YY., Miller, MM., Urban JF., Shi, LZ., Aroian, LV. 2013. An Extensive Comparison of the Effect of Anthelmintic Classes on Diverse Nematodes. PLoS One. 8: e70702. Published online 2013 Jul 15. doi: 10.1371/journal.pone.0070702

Huntley JF, Schallig HD, Kooyman FN, Mackellar A, Jackson F, Smith WD. 1998. IgE antibody during infection with the ovine abomasal nematode, *Teladorsagia circumcincta*: primary and secondary responses in serum and gastric lymph of sheep. Parasite Immunol. 20:565-71.

Immanuel C, Ramanathan A, Balasubramaniyan M, Khatri VK, Amdare NP, Rao DN, Reddy MVR, Perumal K. 2017. Immunoprophylaxis of multi-antigen peptide (MAP) vaccine for human lymphatic filariasis. Immunol Res. 65:729-738. doi: 10.1007/s12026-017-8911-5.

International Helminth Genomes Consortium. 2019 Comparative genomics of the major parasitic worms. Nature Genetics 51:163-174.

Issa, Z., Grant, W.N., Stasiuk, S. Shoemaker, C. B. 2005. Development of methods for RNA interference in the sheep gastrointestinal parasite, *Trichostrongylus colubriformis*. International Journal for Parasitology 35: 935–940.

Jacobs, HJ., Wiltshire, C., Ashman K., Meeusen, ENT. 1999. Vaccination against the gastrointestinal nematode, *Haemonchus contortus*, using a purified larval surface antigen. Vaccine 17: 362-368.

Jarrett, W FH., Jennings, FW., McIntyre, WI. M., Mulligan, W., Sharp, NCC., Urquhart,GM., 1959. Immunological studies on *Dictyocaulus viviparus* infection in calves. Double vaccination with irradiated larvae. Am. J. Vet. Res., 20: 522-526.

Jarrett, W FH., Jennings, FW., McIntyre, WI. M., Mulligan, W., Sharp, NCC., Urquhart,GM., Studies on immunity to *Haemonchus contortus* infection-double vaccination of sheep with irradiated larvae. Am. J. Vet. Res. 22: 186-188.

Jex, A.R., Gasser, R.B., Schwarz, E.M. 2019. Transcriptomic resources for parasitic nematodes of veterinary importance. Trends Parasitol. 35: 72-84

Jones, W.O., Emery, D.L., McClure S.J., Wagland, B.M. 1994. Changes in inflammatory mediators and larval inhibitory activity in intestinal contents and mucus during primary and challenge infections of sheep with *Trichostrongylus colubriformis.* Int. J. Parasitol. 24, 519-525.

Kalyanasundaram, A., Jawahar, S., Ilangopathy, M., Palavesam, A. and Raman, M. (2015). Comparative immunoprophylactic efficacy of Haemonchus contortus recombinant enolase (rHcENO) and Con A purified native glycoproteins in sheep. Experimental Parasitology 154, 98-107.

Karlsson LJE, Greeff JC. Selection response in fecal worm egg counts in the Rylington Merino parasite resistant flock. Aust J Exp Agric. 2006;46(7):809-11.

Keith RK., Bremner, KC. 1973. Immunisation of calves against the nodular worm *Oesophagostomum radiatum*. Res. Vet. Sci. 15; 123-4.

Kelly GA, Kahn LP, Walkden-Brown SWA. 2013. Measurement of phenotypic resilience to gastro-intestinal nematodes in Merino sheep and association with resistance and production variables. Vet. Parasitol. 193:111-117.

Kemper KE, Emery DL, Bishop SC, Oddy H, Hayes BJ, Dominik S, Henshall JM, Goddard ME. 2011. The distribution of SNP marker effects for faecal worm egg count in sheep, and the feasibility of using these markers to predict genetic merit for resistance to worm infections. Genet Res. 2011, 93: 203-219. 10.1017/S0016672311000097.

Kenngott EE, Cole S, Hein WR, Hoffmann U, Lauer U, et al. (2016) Identification of Targeting Peptides for Mucosal Delivery in Sheep and Mice. Mol Pharm 13: 202-210.

Kidane, A., Houdijk, J., Athanasiadou, S., Tolkamp, B., Kyriazakis, I., 2010. Nutritional sensitivity of periparturient resistance to nematode parasites in two breeds of sheep with different nutrient demands. Br. J. Nutr. 104, 1477-1486.

Ingham, A., Reverter, A., Windon, R., Hunt, P., Menzies, M., 2008. Gastrointestinal nematode challenge induces some conserved gene expression changes in the gut mucosa of genetically resistant sheep. Int. J. Parasitol. 38, 431–442.

Jackson F, Greer AW, Huntley J, McAnulty RW, Bartley DJ, Stanley A, Stenhouse L, Stankiewicz M, Sykes AR. 2004. Studies using *Teladorsagia circumcincta* in an *in vitro* direct challenge method using abomasal tissue explants. Vet Parasitol. 124:73-89.

Jacobson, C., Bell, K., Forshaw, D., Besier, Bm 2009. Association between nematode larvae and "low worm egg count diarrhoea" in sheep in Western Australia. *Veterinary Parasitology* 165: 66-73.

Jones, W.O., Emery, D.L., McClure, S.J., Wagland, B.M., 1994. Changes in inflammatory mediators and larval inhibitory activity in intestinal contents and mucus during primary and challenge infections of sheep with *Trichostrongylus colubriformis*. Int. J. Parasitol. 24, 519 525.

Kemp JM, Robinson NA, Meeusen EN, Piedrafita DM. 2009. The relationship between the rapid rejection of *Haemonchus contortus* larvae with cells and mediators in abomasal tissues in immune sheep. Int J Parasitol. 2009 Dec;39(14):1589-94. doi: 10.1016/j.ijpara.05.015.

Khatoon N, Ojha R, Mishra A, Prajapati VK. 2018. Examination of antigenic proteins of *Trypanosoma cruzi* to fabricate an epitope-based subunit vaccine by exploiting epitope mapping mechanism. Vaccine. 2018;36(42):6290-6300. doi:10.1016/j.vaccine.09.004.

Kotze, A.C., Bagnall, N.H. 2006. RNA interference in *Haemonchus contortus*: suppression of beta-tubulin gene expression in L3, L4 and adult worms in vitro. Mol. Biochem. Parasitol. 145, 101e110.

Kunkel EJ, Butcher EC (2003) Plasma-cell homing. Nat Rev Immunol 3: 822-829.

Lacey E., Redwin J.M., Gil l.J.H., Demargherit i.V.M., Waller P.J. 1990. A larval development assay for the simultaneous detection of broad spectrum anthelmintic resistance. In: Boray J.C., Martin P.J., Roush R.T., editors. "Resistance of Parasites to Antiparasitic Drugs". MSD Agvet; New Jersey. pp. 177–184.

Laing, R., Kikuchi, T., Martinelli, A., Tsai, I.J., Beech, R.N., et al. 2013. The genome and transcriptome of *Haemonchus contortus*, a key model parasite for drug and vaccine discovery. Genome Biol. 14: R88.

Lane, J., Jubb, T., Shephard, R., Webb-Ware, J., Fordyce, G., 2015. MLA Final Report: Priority list of endemic diseases for the red meat industries. Meat and Livestock Australia, Sydney, Australia. http://www.wormboss.com.au/files/pages/worms/roundworms/the-cost-of-roundworms/B.AHE.0010_Final_Report.pdf.

Larsen, JWA., Anderson, N. 2000. The relationship between the rate of intake of trichostrongylid larvae and the occurrence of diarrhoea and breech soiling in adult Merino sheep. Aust. Vet. J., 78: 112-116.

Larsen, JWA., Anderson N., Vizard AL. et al. 1994. Diarrhoea in Merino ewes during winter: association with Trichostrongylid larvae. Aust. Vet. J. 71:365-372.

Larsen, JWA., Vizard, AL., Webb-Ware, JK., Anderson, N. 1995. Diarrhoea due to trichostrongylid larvae in Merino sheep during winter: repeatability and differences between bloodlines. Aust. Vet. J. 72:196-197.

Larsen JWA, Anderson NA, Vizard AL. 1999. The pathogenesis and control of diarrhoea and breech soiling in adult Merino sheep. Int. J. Parasitol. 29:893-902.

Lascelles, AK., Eagleson, G., Beh, KJ., Watson DL. 1989. Significance of Freund's adjuvant/antigen injection granuloma in the maintenance of serum antibody response.Vet. Immunol. Immunopathol..22:15-27.

Laurenson YCSM, Kyriazakis I, Bishop SC. 2013. Can we use genetic and genomic approaches to identify candidate animals for targeted selective treatment? Vet Parasitol. 197: 379-383.

Laurenson YCSM, Kahn LP. A mathematical model to predict the risk arising from the pasture infectivity of four nematode species in Australia. Animal Production Sci. 2018; 58: 1504-1514

Lee SE, Kim SY, Jeong BC, Kim YR, Bae SJ, et al. (2006) A bacterial flagellin, *Vibrio vulnificus* FlaB, has a strong mucosal adjuvant activity to induce protective immunity. Infect Immun 74: 694-702.

Le Jambre, L.F., Windon, R.G., Smith, W.D., 2008. Vaccination against *Haemonchus contortus*: performance of native parasite gut membrane glycoproteins in Merino lambs grazing contaminated pasture. Vet. Parasitol. 153, 302–312.

Le Jambre LF, Dobson RJ, Lenane IJ, Barnes EH. Selection for anthelmintic resistance by macrocyclic lactones in *Haemonchus contortus*. Int. J. Parasitol. 1999. 29: 1101–1111

Leathwick DM et al. 2000

Leathwick DM, Besier RB. The management of anthelmintic resistance in grazing ruminants in Australasia – strategies and experiences. Vet. Parasitol. 2014; 204: 44- 54.

Leathwick, D.M., Miller, C.M., Fraser, K., 2015. Selection for anthelmintic resistant Teladorsagia circumcincta in pre-weaned lambs by treating their dams with long-acting moxidectin injection. Int. J. Parasitol. Drugs Drug Resist. 5, 209-214.

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

Le Feuvre A, Woodgate RG, Carmichael I, Bailey A , Love S, Campbell N, Besier B, Page P. WormBoss. Proc. Aust. Sheep Veterinarians Ann. Conf. 2005; 15:85-88.

Levine ND. Weather, climate, and the bionomics of ruminant nematode larvae. Advances in Vet. Sci. 1963, 8; 215-261.

Levine, ND. Nematode parasites of domestic animals and of man (2nd Edition). 1980; Burgess Publishing Company, Minneapolis.

Lew-Tabor AE, Rodriguez Valle M. (2016) A review of reverse vaccinology approaches for the development of vaccines against ticks and tick borne diseases. Ticks Tick Borne Dis. 7, 573-85.

Lino CA, Harper JC, Carney JP, Timlin JA. 2018. Delivering CRISPR: a review of the challenges and approaches. Drug Delivery 25:1, 1234-1257

Liu P, Wang ZQ, Liu RD, Jiang P, Long SR, et al. (2015) Oral vaccination of mice with *Trichinella spiralis* nudix hydrolase DNA vaccine delivered by attenuated *Salmonella* elicited protective immunity. Exp Parasitol 153: 29-38.

Lok JB, Shao H, Massey HC, Li X. 2017. Transgenesis in *Strongyloides* and related parasitic nematodes: historical perspectives, current functional genomic applications and progress towards gene disruption and editing. Parasitology 144(3):327–42.

Maass D.R., Harrison G.B., Grant W.N., Hein W.R., Shoemaker C.B. 2009. Intraspecific Epitopic Variation in a Carbohydrate Antigen Exposed on the Surface of Trichostrongylus colubriformis Infective L3 Larvae. PLoS Pathol. 5: e1000597. doi:10.1371/journal.ppat.1000597

Mackinnon MJ, Meyer K, Hetzel DJS. Genetic variation and covariation for growth, parasite resistance and heat tolerance in tropical cattle. Livestock Production Science. 1991;27:105-22.

MartÍNez-Valladares M, Vara-Del RÍO MP, Cruz-Rojo MA, Rojo-VÁZquez FA. Genetic resistance to Teladorsagia circumcincta: IgA and parameters at slaughter in Churra sheep. *Parasite Immunol.* 2005;27(6):213-218.

Matthews, J.B., Geldhof, P., Tzelos, T., Claerebout, E., 2016. Progress in the development of subunit vaccines for gastrointestinal nematodes of ruminants. Parasite Immunol. 38, 744-753.

Makepeace BL, Jensen SA, Laney SJ, Nfon CK, Njongmeta LM, Tanya VN, Williams SA, Bianco AE, Trees AJ. 2009. Immunisation with a multivalent, subunit vaccine reduces patent infection in a natural bovine model of onchocerciasis during intense field exposure. PLoS Negl Trop Dis. 3:e544. doi:10.1371/journal.pntd.0000544

Mayer, L., Shao, L. 2004. Therapeutic potential of oral tolerance. Nat Rev Immunol. 4:407-19.

McClure, S. 2000. Sheep immunity to gastrointestinal nematode parasites-review 2000. http: //www. csiro.au/proprietaryDocuments/McClure_Review2000. pdf.

McClure, SJ, 2008. Mucosal delivery of native and recombinant protein vaccines against Trichostrongylus colubriformis. Int. J. Parasitol. 39:599-606. DOI: 10.1016/j.ijpara.2008.09.010.

McClure S.J., Davey, R.J., Lloyd, J.B., Emery, D.L. 1995. Depletion of IFN-γ, CD8+ or Tcr γδ+ cells in vivo during primary infection with an enteric parasite (Trichostrongylus colubriformis) enhances protective immunity. Immunol. Cell.Biol. 73, 552-555.

McClure, SJ., Emery, DL. 2007. *Trichostrongylus colubriformis* and *Haemonchus contortus* infections in light bodyweight Merino lambs. Aust. Vet. J., 85;437-445.

McClure, SJ., Emery, DL., TBendixsen, T., Davey, RJ. 1998. Attempts to generate immunity against T*richostrongylus colubriformis* and *Haemonchus contortus* in young lambs by vaccination with viable parasites. Int. J. Parasitol., 28: 739-46.

McClure, S.J., Emery, D.L. and Husband, A.J., 1994. The effects of adjuvants and routes of delivery on the development of mucosal immune responses to ovalbumin in sheep. Reg. Immunol., 6: 210-217.

McClure, S.J., McClure, T.J., Emery, D.L., 1999. Effects of molybdenum intake on primary infection and subsequent challenge by the nematode parasite *Trichostrongylus colubriformis* in weaned Merino lambs. Res. Vet Sci. 67: 17-22.

McClure S.J., Emery, D.L., Wagland, B.M., Jones, W.O. 1992. A serial study of rejection of T*richostrongylus colubriformis* by immune sheep. Int. J. Parasitol. 22, 227-234.

McKellar Q. 1993. Interactions of *Ostertagia* species with their bovine and ovine hosts. Int. J. Parasitol. 23:451-462.

McNeilly TN, Naylor SW, Mahajan A, Mitchell MC, McAteer S, et al. 2008. *Escherichia coli* O157:H7 colonization in cattle following systemic and mucosal immunization with purified H7 flagellin. Infect Immun 76: 2594-2602.

McNeilly TN, Frew D, Burgess STG, Wright H, Bartley DJ, Bartley Y, Nisbet AJ. 2017. Niche-specific gene expression in a parasitic nematode; increased expression of immunomodulators in *Teladorsagia circumcincta* larvae derived from host mucosa. Sci Rep. ;7:7214. doi: 10.1038/s41598-017-07092-0

McNeilly, T.N., McClure, S.J., Huntley, J.F., 2008. Mucosal immunity in sheep and implications for mucosal vaccine development. Small Ruminant Res. 76, 83–91.

McNeilly TN, Nisbet AJ. 2014. Immune modulation by helminth parasites of ruminants: implications for vaccine development and host immune competence. Parasite.21:51. doi: 10.1051/parasite/2014051.

McRae, KM., Stear, MJ., Good. B., Keane, OM. 2015. The host immune response to gastrointestinal nematode infection in sheep. Parasite immunology. 37: 605-613.

Meeusen E, Balic A. Do Eosinophils have a Role in the Killing of Helminth Parasites? *Parasitol Today.* 2000;16:95-101.

Meeusen E et al., 2013. New approaches to innate immunity in livestock and the potential for manipulation . MLA report B.BSC.0321. https://www.mla.com.au/research-and-development/search-rd-reports/final-report-details/Animal-Health-and-Biosecurity/New-approaches-to-innate-immunity-in-livestock-and-the-potential-for-manipulation-review/206.

Menon R, Gasser RB, Mitreva M, Ranganathan S. 2012. An analysis of the transcriptome of *Teladorsagia circumcincta*: its biological and biotechnological implications. BMC Genomics.13 Suppl 7:S10. doi: 10.1186/1471-2164-13-S7-S10.

Merrifield M, Hotez PJ, Beaumier CM, Gillespie P, Strych U, Hayward T, Bottazzi ME. 2016. Advancing a vaccine to prevent human schistosomiasis. Vaccine. 34:2988-2991.

Miller, H.R.P., 1984. The protective mucosal response against gastrointestinal nematodes in ruminants and laboratory animals. Vet. Immun. Immunopath. 6, 167-259.

Miller, H.R.P. 1996. Mucosal mast cells and the allergic response against nematode parasites. Vet Immunol. mmunopathol. 54, 331-336.

MLA, 2017. B.AHE.0054. Estimating the cost of immunity to gastro-intestinal nematodes in meat sheep differing genetically in resistance and resilience to infection. https://www.mla.com.au/research-and-development/search-rd-reports/final-report-details/Animal-Health-and-Biosecurity/Estimating-the-cost-of-immunity-to-gastro-intestinal-nematodes-in-meat-sheep-differing-genetically-in-resistance-and-resilien/3502 .

Morein B, Hu KF, Abusugra I (2004) Current status and potential application of ISCOMs in veterinary medicine. Adv Drug Deliv Rev 56: 1367-1382.

Morgan E, Charlier J, Hendrickx G, et al. Global Change and Helminth Infections in Grazing Ruminants in Europe: Impacts, Trends and Sustainable Solutions. *Agriculture.* 2013;3(3):484-502.

Morris RS, Anderson NA, McTaggart IK. 1977.An economic analysis of two schemes for the control of helminthiasis in breeding ewes. Vet. Parasitol. 3:349-363.

Morris C, Vlassoff A, Bisset S, Baker R, West C, Hurford A. 1997.Responses of Romney sheep to selection for resistance or susceptibility to nematode infection. Anim Sci. 64:319 - 29.

Mrode R, Tarekegn GM, Mwacharo JM, Djikeng A. Genomic selection for small ruminants in developed countries: how applicable for the rest of the world? Animal. 2018;12:1333-40.

Mugambi JM, Wanyangu SW, Bain RK, Owango MO, Duncan JL, Stear MJ. Response of Dorper and Red Maasai lambs to trickle *Haemonchus contortus* infections. Res Vet Sci. 1996;61:218-221.

Murphy L, Eckersall PD, Bishop SC, et al. 2010. Genetic variation among lambs in peripheral IgE activity against the larval stages of *Teladorsagia circumcincta. Parasitology.*137):1249-1260.

Mutwiri, G., Bateman, C., Baca-Estrada, M.E., Snider, M., Griebel, P., 2000. Induction of immune responses in newborn lambs following enteric immunization with a human adenovirus vaccine vector. Vaccine 19, 1284–1293.

Myers, G. S., Parker, D., Al-Hasani, K., Kennan, R. M., Seemann, T., Ren, Q. et al., 2007. Genome sequence and identification of candidate vaccine antigens from the animal pathogen *Dichelobacter nodosus.* Nat. Biotechnol., 25, 569-75.

Nagaraj SH, Gasser RB, Nisbet AJ, Ranganathan S. 2008. *In silico* analysis of expressed sequence tags from *Trichostrongylus vitrinus* (Nematoda): comparison of the automated ESTExplorer workflow platform with conventional database searches. BMC Bioinformatics. 9 Suppl 1:S10. doi: 10.1186/1471-2105-9-S1-S10

Neill DR , Wong SH , Bellosi A , Flynn RJ , Daly M , Langford TK , Bucks C , Kane CM , Fallon PG , Pannell R , Jolin HE , McKenzie AN. 2010. Nuocytes represent a new innate effector leukocyte that mediates type-2 immunity. Nature. 464 (7293): 1367–70. doi:10.1038/nature0890.

Neutra MR, Mantis NJ, Kraehenbuhl JP (2001) Collaboration of epithelial cells with organized mucosal lymphoid tissues. Nat Immunol 2: 1004-1009.

Nieuwhof GJ, Bishop SC. Costs of the major endemic diseases of sheep in Great Britain and the potential benefits of reduction in disease impact. *Anim Sci.* 2005;81:23-29.

Nisbet AJ, Gasser RB. 2004. Profiling of gender-specific gene expression for *Trichostrongylus vitrinus* (Nematoda: Strongylida) by microarray analysis of expressed sequence tag libraries constructed by suppressive-subtractive hybridisation. Int J Parasitol. 34:633-43

Nisbet AJ, Knox DP, McNair CM, Meikle LI, Smith SK, Wildblood LA, Matthews JB. 2009. Immune recognition of the surface associated antigen, Tc-SAA-1, from infective larvae of *Teladorsagia circumcincta*. Parasite Immunol. 31:32-40. doi:10.1111/j.1365-3024.2008.01070.x.

Nisbet, A.J., McNeilly, T.N., Wildblood, L.A., Morrison, A.A., Bartley, D.J., Bartley, Y., Longhi, C., et al. 2013. Successful immunization against a parasitic nematode by vaccination with recombinant proteins. Vaccine 31, 4017-4023.

Nisbet AJ, Meeusen EN, González JF, Piedrafita DM. 2016. Immunity to *Haemonchus contortus* and Vaccine Development. Adv Parasitol. 93:353-96. doi: 10.1016/bs.apar.2016.02.011.

Nisbet, A.J., McNeilly, T.N., Greer, A.W., Bartley, Y., Oliver, E.M., Smith, S., Palarea-Albaladejo, J., Matthews, J.B., 2016b. Protection of ewes against *Teladorsagia circumcincta* infection in the periparturient period by vaccination with recombinant antigens. Vet. Parasitol. 228, 130-136.

Nisbet, A.J., Redmond, D.L., Matthews, J.B., Watkins, C., Yaga, R., Jones, J.T., Nath, M., Knox, D.P., 2008. Stage-specific gene expression in *Teladorsagia circumcincta* (Nematoda: Strongylida). International Journal for Parasitology 38, 829-838.

Nisbet, A.J., McNeilly, T.N., Price, D.R.G., Oliver, E.M., Bartley, Y., Mitchell, M., Palarea-Albaladejo, J., Matthews, J.B. 2019. The rational simplification of a recombinant cocktail vaccine to control the parasitic nematode *Teladorsagia circumcincta.* International Journal for Parasitology https://doi.org/10.1016/j.ijpara.2018.10.006

O'Connor LJ, Walkden-Brown SW, Kahn LP. 2006. Ecology of the free-living stages of major trichostrongylid parasites of sheep.Vet. Parasitol. 42: 1-15.

O'Handley, R. 2013. Slow-release capsule for reducing winter scours in sheep – Stage: proof of concept. MLA report B.AHE.0088. https://www.mla.com.au/research-and-development/search-rd-reports/final-report-details/Animal-Health-and-Biosecurity/Slow-release-capsule-for-reducing-winter-scours-in-sheep-Stage-proof-of-concept/167

Oliveira FM, Coelho IE, Lopes MD, Taranto AG, Junior MC, Santos LL, Villar JA, Fonseca CT, Lopes DD. 2106. The Use of Reverse Vaccinology and Molecular Modeling Associated with Cell Proliferation Stimulation Approach to Select Promiscuous Epitopes from *Schistosoma mansoni*. Appl Biochem Biotechnol. 179:1023-40.

Osterbye T, Buus S (2015) Automated High-Throughput Mapping of Linear B-Cell Epitopes Using a Statistical Analysis of High-Density Peptide Microarray Data. Methods Mol Biol 1348: 215-228.

Pernthaner, A., Shaw ,R.J., McNeill ,M.M., Morrison, L., Hein, W.R., 2005a. Total and nematode-specific IgE responses in intestinal lymph of genetically resistant and susceptible sheep during infection with *Trichostrongylus colubriformis*. Vet. Immunol. Immunopath., 104, 69–80.

Pernthaner, A., Cole, S.A., Morrison, L., Hein, W.R., 2005b. Increased expression of interleukin-5 (IL-5), IL-13, and tumor necrosis factor alpha genes in intestinal lymph cells of sheep selected for enhanced resistance to nematodes during infection with *Trichostrongylus colubriformis*. Inf. Immun. 73, 2175–2183.

Pernthaner, A., Cole, SA., Morrison, L., Green, R., Shaw, RJ., Hein, WR.,2006. Cytokine and antibody subclass responses in the intestinal lymph of sheep during repeated experimental infections

with the nematode parasite *Trichostrongylus colubriformis*. Vet. Immunol. Immunopathol. 114: 135–148.

Piedrafita D, Preston S, Kemp J, de Veer M, Sherrard J, Kraska T, Elhay M, Meeusen E. 2013. The effect of different adjuvants on immune parameters and protection following vaccination of sheep with a larval-specific antigen of the gastrointestinal nematode, *Haemonchus contortus*. PLoS One. 2013 Oct 21;8(10):e78357.

Pfeffer, A., Douch, P.G.C., Shaw, R.J., Gatehouse, T.K., Rabel, B., Green, R.S., Shirer, C.L., Jonas, W.E., Bisset, S. 996. Sequential cellular and humoral responses in the abomasal mucosa and blood of Romney sheep dosed with *Trichostrongylus axei*. Int. J. Parasitol. 26, 765-773.

Pizza M, Scarlato V, Masignani V, Giuliani MM, Aricò B, Comanducci M, Jennings GT., et al. 2002. Identification of vaccine candidates against serogroup B meningococcus by whole-genome sequencing. Science. 287:1816-20.

Pompa-Mera EN, Yepez-Mulia L, Ocana-Mondragon A, Garcia-Zepeda EA, Ortega-Pierres G, et al. 2011. T*richinella spiralis*: intranasal immunization with attenuated *Salmonella enterica* carrying a gp43 antigen-derived 30mer epitope elicits protection in BALB/c mice. Exp Parasitol 129: 393-401.

Powell RH, Behnke MS. 2017. WRN conditioned media is sufficient for *in vitro* propagation of intestinal organoids from large farm and small companion animals. Biol Open 6:698-705.

Premier RR, Jacobs HJ, Lofthouse SA, Sedgmen BJ, Meeusen EN (2004) Antibody isotype profiles in serum and circulating antibody-secreting cells following mucosal and peripheral immunisations of sheep. Vet Immunol Immunopathol 98: 77-84.

Prichard RK, Hall CA, Kelly JD, Martin ICA Donald AD. 1980.The problem of anthelmintic resistance in nematodes. Aust. Vet. J. 56: 239-251

Rappuoli R, Bottomley MJ, D'Oro U, Finco O, De Gregorio E. 2016. Reverse vaccinology 2.0: Human immunology instructs vaccine antigen design. J Exp Med. 213:469-81.

Redman E., Grillo, V., Saunders, G., Packard, E., Jackson, F., Berriman, M., Gilleard, JS. 2008. Genetics of mating and sex determination in the parasitic nematode *Haemonchus contortus.* Genetics 180: 1877–1887

Redmond, DL., Knox, D.P. 2004. Protection studies in sheep using affinity-purified and recombinant cysteine proteinases of adult *Haemonchus contortus*. Vaccine 22, 4252-4261.

Redmond, DL., Knox, D.P. 2006. Further protection studies using recombinant forms of *Haemonchus contortus* cysteine proteinases. Parasite Immunology 28, 213-219.

Redmond, DL., Smith, SK., Halliday, A., Smith, WD., Jackson. F., Knox. DP., Matthews, JB. 2006. An immunogenic cathepsin F secreted by the parasitic stages of *Teladorsagia circumcincta.* Int J Parasitol. 36:277-86.

Reeve, I., Walkden-Brown S. 2014. Benchmarking Australian sheep parasite control (WP499). Longitudial survey report. MLA report B.AHE.0069. *https://www.mla.com.au/download/finalreports?itemId=2080*

Riggio V, Matika O, Pong-Wong R, Stear MJ, Bishop SC. Genome-wide association and regional heritability mapping to identify loci underlying variation in nematode resistance and body weight in Scottish Blackface lambs. *Heredity (Edinb).* 2013;110(5):420-429.

Roberts, B., Antonopoulos, A., Haslam, S.M., Dicker, A.J., McNeilly, T.N., Johnston, S.L., Dell, A., Knox, D.P., Britton, C. 2013. Novel expression of *Haemonchus contortus* vaccine candidate aminopeptidase H11 using the free-living nematode *Caenorhabditis elegans*. Vet Res 44, 111.

Roeber F, Kahn LP. 2014. The specific diagnosis of gastrointestinal nematode infections in livestock: Larval culture technique, its limitations and alternative DNA-based approaches. Vet. Parasitol. 205:619-628

Rowe, A., Gondro, C., Emery, D.L., Sangster, N., 2009. Sequential microarray to identify timing of molecular responses to *Haemonchus contortus* infection in sheep. Int J. Parasitol. 161, 76–87.

Sakkas, P., Houdijk, J.G., Athanasiadou, S., Kyriazakis, I., 2012. Sensitivity of periparturient breakdown of immunity to parasites to dietary protein source. J. Anim. Sci. 90, 3954-3962.

Salisbury JR, Arundel JH. 1970. Peri-parturient deposition of nematode eggs by ewes and residual pasture contamination as sources of infection for lambs. Aust. Vet. J. 1970; 46: 523-529.

Sallé G, Laing R, Cotton JA, Maitland K, Martinelli A, Holroyd N, Tracey A, Berriman M et al. 2018. Transcriptomic profiling of nematode parasites surviving vaccine exposure. Int J Parasitol. 48:395-402. doi: 10.1016/j.ijpara.2018.01.004.

Samarasinghe, B., Knox, D. P. and Britton, C. 2011. Factors affecting susceptibility to RNA interference in *Haemonchus contortus* and in vivo silencing of an H11 aminopeptidase gene. Int J. Parasitol.41:51–59.

Sargison, N.D., Bartram, D.J., Wilson, D.J., 2012. Use of a long-acting injectable formulation of moxidectin to control the periparturient rise in faecal Teladorsagia circumcincta egg output of ewes. Vet Parasitol. 189, 274-283.

Sato T, van Es JH, Snippert HJ, Stange DE, Vries RG, van den Born M, Barker N, Shroyer NF, van de Wetering M, Clevers H. 2011. Paneth cells constitute the niche for Lgr5 stem cells in intestinal crypts. Nature 469:415–418

Sayers G, Good B, Hanrahan JP, Ryan M, Angles JM, Sweeney T. 2005.Major histocompatibility complex *DRB1* gene: its role in nematode resistance in Suffolk and Texel sheep breeds. Parasitology. 131:403-9.

Schallig, H.D., van Leeuwen, M.A., Cornelissen, A.W. (1997a). Protective immunity induced by vaccination with two Haemonchus contortus excretory secretory proteins in sheep. Parasite Immunology 19, 447-453.

Schwarz, E.M., Korhonen, P.K., Campbell, B.E., Young, N.D., Jex, A.R., Jabbar, A. et al., 2013. The genome and developmental transcriptome of the strongylid nematode *Haemonchus contortus.* Genome Biol. 14, R89.

Scott, I, Pomroy WE, Kenyon PR, Smith G, Adlington B, Moss A. 2013. Lack of efficacy of monepantel against *Teladorsagia circumcincta* and T*richostrongylus colubriformis*. Vet. Parasitol. 2013; 198:166 – 171.

Seaton, D.S., Jackson, F., Smith, W.D., Angus, K.W., 1989. Development of immunityto incoming radiolabelled larvae in lambs continuously infected with *Ostertagia circumcincta*. Res. Vet. Sci. 46, 241–246.

Shaw, R.J., Morris, C.A., Green, R.S., Wheeler, M., Bisset, S.A., Vlassoff, A.,Douch, P.G.C., 1999. Genetic and phenotypic parameters for T*richostrongylus colubriformis*-specific immunoglobulin E and its relationships with anti-*Trichostrongylus colubriformis* antibody, immunoglobulin G1, faecal egg count and body weight traits in grazing Romney. Livest. Prod. Sci. 58: 25–32.

Shaw, R., Morris, C., Wheeler, M., Tate, M. and Sutherland, I. 2012. Salivary IgA: a suitable measure of immunity to gastrointestinal nematodes in sheep. Veterinary Parasitology 186; 109–117.

Shaw, RJ., Morris, CA., Wheele,r M. 2013. Genetic and phenotypic relationships between carbohydrate larval antigen (CarLA) IgA, parasite resistance and productivity in serial samples taken from lambs after weaning. Int. J. Parasitol. 43:661-7.

Shaw, R., Pfeffer, A. and Bischof, R. 2009. Ovine IgE and its role in immunological protection and disease. Vet.

Immunol. Immunopath. 132: 31–40.

Shears RK, Bancroft AJ, Hughes GW, Grencis RK, Thornton DJ. 2018. Extracellular vesicles induce protective immunity against *Trichuris muris.* Parasite Immunology 40:e12536.

Simpson HV. Pathophysiology of Abomasal Parasitism: Is the Host or Parasite Responsible? The Vet. J. 2000; 160: 177–191.

Singleton, D.R., Stear, M.J., Matthews, L., 2011. A mechanistic model of developing immunity to *Teladorsagia circumcincta* infection in lambs. Parasitology 138, 322-332.

Sinski E, Bairden K, Duncan JL, et al. 1995. Local and plasma antibody-responses to the parasitic larval stages of the abomasal nematode *Ostertagia circumcincta*. *Vet Parasitol.* 59:107-118.

Smith, W.D., Jackson, F., Jackson, E., Williams, J., 1983. Local immunity and *Ostertagia circumcincta*: changes in the gastric lymph of immune sheep after a challenge infection. J. Comp. Path. 93, 479–488.

Smith, W.D., Jackson, F., Jackson, E., Williams, J., 1985. Age immunity to Ostertagia circumcincta: comparison of the local immune responses of 4 1/2- and 10-month-old lambs. J. Comp. Pathol. 95, 235-245.

Smith, W.D., Jackson, F., Jackson, E., Graham, R., Williams, J., 1986. Transfer of immunity to Ostertagia circumcincta and IgA memory between identical sheep by lymphocytes collected from gastric lymph. Res. Vet. Sci. 41, 300-306.

Smith, W.D., Jackson, F., Graham, R., Jackson, E., Williams, J., 1987. Mucosal IgA production and lymph cell traffic following prolonged low level infections of Ostertagia circumcincta in sheep. Res. Vet. Sci. 43, 320-326.

Spithill, T.W., Dalton, J.P. 1998. Progress in development of liver fluke vaccines. Parasitology Today, 14; 224–228.

Stankiewicz, M., Cabaj, W., Pernthaner, A., Jonas, W., Rabel, B. 1996. Drug-abbreviated infections and development of immunity against Trichostrongylus colubriformis in sheep. Int. J. Parasitol. 26:97-103

Stanley, AC., Buxton, D., Innes, EA., Huntley, JF. 2004. Intranasal immunisation with *Toxoplasma gondii* tachyzoite antigen encapsulated into PLG microspheres induces humoral and cell-mediated immunity in sheep. Vaccine 22: 3929–3941.

Stewart, D.F., 1955. 'Self-cure' in nematode infections of sheep. Nature 176, 1273-1274.

Stear MJ, Bishop SC, Doligalska M, et al. 1995. Regulation of egg production, worm burden, worm length and worm fecundity by host responses in sheep infected with *Ostertagia circumcincta. Parasite Immunol.* 17:643-652.

Stear MJ, Bishop SC. 1999. The curvilinear relationship between worm length and fecundity of *Teladorsagia circumcincta. Int J Parasitol.*29:777-780.

Stear MJ, Bairden K, Bishop SC, et al. 1998. The processes influencing the distribution of parasitic nematodes among naturally infected lambs. *Parasitology.* 1998;117:165-171.

Stear MJ, Bishop SC, Henderson NG, Scott I. 2003. A Key Mechanism of Pathogenesis in Sheep Infected with the Nematode *Teladorsagia circumcincta. Anim Health Res Rev.*;4:45-52.

Stear MJ, Abuargob O, Ben Othman M, et al. 2006. Variation among faecal egg counts following natural nematode infection in Scottish Blackface lambs. *Parasitology.*132:275-280.

Stear MJ, Henderson NG, Kerr A, et al. 2002. Eosinophilia as a marker of resistance to *Teladorsagia circumcincta* in Scottish Blackface lambs. *Parasitology.*124:553-560.

Stear MJ, Innocent GT, Buitkamp J. 2005. The evolution and maintenance of polymorphism in the major histocompatibility complex. Vet Immunol Immunopathol. 108:53-7.

Stear MJ, Park M, Bishop SC. 1996. The key components of resistance to *Ostertagia circumcincta* in lambs. *Parasitol Today.* 12:438-441.

Stear MJ, Tierney TJ, Baldock FC, Brown SC, Nicholas FW, Rudder TH. 1988. Class I antigens of the bovine major histocompatibility system are weakly associated with variation in faecal worm egg counts in naturally infected cattle. Anim Genet. 19:115-22.

Stear MJ, Bairden K, Duncan JL, et al. 2000. The influence of relative resistance and urea-supplementation on deliberate infection with *Teladorsagia circumcincta* during winter. *Vet Parasitol.* 94:45-54.

Stewart, D. J., Clark. B. L., Emery. D. L., Peterson, J. E., Kortt, A. A. 1985. The phenomenon of cross protection against footrot induced by vaccination of sheep with *Bacteroides nodosus* vaccines. ln “Footrot of Ruminants”. D.J.Stewart, J . E. Peterson, N. M. McKern and D. L. Emery (eds). CSIRO/Australian Wool Corporation, Melbourne. pp. 185-192.

Stewart, M.J., McClure, S.J., Rothwell, T.L.W., Emery, D.L. 1995. Changes in the enteric innervation of the sheep during primary or secondary immune responses to the nematode *Trichostrongylus colubriformis.* Regional Immunology 6: 460-470.

Strain SAJ, Bishop SC, Henderson NG, Kerr A, McKellar QA, Mitchell S, et al. The genetic control of IgA activity against *Teladorsagia circumcincta* and its association with parasite resistance in naturally infected sheep. Parasitology. 2002;124.

Stringfellow F.1986. Cultivation of *Haemonchus contortus* (Nematoda: Trichostrongylidae) from infective larvae to the adult male and the egg-laying female. J. Parasitol. 72:339-345.

Stutzer C, Richards SA, Ferreira M, Baron S, Maritz-Olivier C., 2018. Metazoan parasite vaccines: Present status and future prospects. Front. Cell Infect. Microbiol. 8, 67.

Tabor A. 2019. SophisTICKated anti-tick vaccines- cattle tick and Australian paralysis tick. Proceedings of the UK and International Veterinary Vaccinology Network Conference, London 9-10th January 2019.

Taylor, S.M., Edgar, H., Kenny, J., 1993. Prophylactic efficacy of moxidectin for periparturient ewes and mid-summer lambs. Vet. Rec. 133, 270-271.

Taylor, S.M., Kenny, J., Edgar, H.W., Ellison, S., Ferguson, L., 1997. Efficacy of moxidectin, ivermectin and albendazole oral drenches for suppression of periparturient rise in ewe worm egg output and reduction of anthelmintic treatment for lambs. Vet. Record 141, 357-360.

Tremain, S.A., Emery, D.L. 1994. Myoelectric activity of the small intestine during parasite rejection. 30th Annual Scientific Meeting of the Australian Society for Parasitology p 43.

Tritten, L., Burkman, E., Moorhead, A., Satti, M., Geary, J., Mackenzie, C., Geary, T. 2014. Detection of circulating parasite-derived microRNAs in filarial infections. Plos Negl. Trop. Dis 8:e2971.

Tsuji N, Miyoshi T, Islam MK, Isobe T, Yoshihara S, et al. (2004) Recombinant Ascaris 16-Kilodalton protein-induced protection against *Ascaris suum* larval migration after intranasal vaccination in pigs. J. Infect. Dis. 190: 1812-1820.

Tsuji N, Suzuki K, Kasuga-Aoki H, Isobe T, Arakawa T, et al. (2003) Mice intranasally immunized with a recombinant 16-kilodalton antigen from roundworm Ascaris parasites are protected against larval migration of *Ascaris suum*. Infect. Immun. 71: 5314-5323.

Tsuji N, Suzuki K, Kasuga-Aoki H, Matsumoto Y, Arakawa T, et al. (2001) Intranasal immunization with recombinant *Ascaris suum* 14-kilodalton antigen coupled with cholera toxin B subunit induces protective immunity to *A. suum* infection in mice. Infect. Immun. 69: 7285-7292.

Tzelos, T., Matthews, J.B., Whitelaw, B., Knox, D.P. 2015. Marker genes for activation of the RNA interference (RNAi) pathway in the free-living nematode *Caenorhabditis elegans* and RNAi development in the ovine nematode *Teladorsagia circumcincta*. J. Helminthol. 89: 208e216.

Urban JF, Katona IM, Paul WE, Finkelman FD.1991. Interleukin 4 is important in protective immunity to a gastrointestinal nematode infection in mice. *Proceedings of the National Academy (USA).* 88:5513-5517.

Urquhart GM, Armour J, Duncan JL, Dunn AM, Jennings FM. Veterinary Parasitology (2nd edition). 1996; Blackwell Science.

Van der Stede Y, Cox E, Van den broeck W, Goddeeris BM (2001) Enhanced induction of the IgA response in pigs by calcitriol after intramuscular immunization. Vaccine 19: 1870-1878.

van Egmond M, Damen CA, van Spriel CA, Vidarsson G, van Garderen E, van de Winkel JGJ. 2001.IgA and the IgA Fc receptor. *Trends Immunol.* 22:205-211.

Van Gramberg, JL., de Veer, MJ., O'Hehir, RE., Meeusen, ENT., Bischof, RJ. 2012. Induction of Allergic Responses to Peanut Allergen in Sheep. PLoS One. 7: e51386.

van Houtert, M.F.J., Barger, I.A., Steel, J.W., Windon, R.G., Emery, D.L. 1995. Effects of dietary protein intake on responses of young sheep to infection with *Trichostrongylus colubriformis*. Vet. Parasitol. 56, 163-180.

van Pinxteren LA, Bruce MG, Campbell I, Wood A, Clarke CJ, et al. (1999) Effect of oral rotavirus/iscom vaccines on immune responses in gnotobiotic lambs. Vet Immunol Immunopathol 71: 53-67.

Verkuylen AJ, Frenkel MJ, Savin KW, Dopheide TA, Ward CW. 1993. Characterization of the mRNA encoding a proline-rich 37-kilodalton glycoprotein from the excretory-secretory products of *Trichostrongylus colubriformis*. Mol. Biochem Parasitol. 58:325-32.

Vervelde, L., Van Leeuwen, M.A., Kruidenier, M., Kooyman, F.N., Huntley, J.F., Van Die, I. and Cornelissen, A.W. (2002). Protection studies with recombinant excretory/secretory proteins of *Haemonchus contortu*s. Parasite Immunology 24, 189-201.

Vilte DA, Larzabal M, Garbaccio S, Gammella M, Rabinovitz BC, et al. (2011) Reduced faecal shedding of Escherichia coli O157:H7 in cattle following systemic vaccination with gamma-intimin C(2)(8)(0) and EspB proteins. Vaccine 29: 3962-3968.

von Moltke J, Ji M, Liang HE, Locksley RM. 2016. Tuft-cell-derived IL-25 regulates an intestinal ILC2-epithelial response circuit. Nature 529:221-225.

Wagland, B.M., Emery, D.L., McClure, S.J. 1996. Studies on the host-parasite relationship between *Trichostrongylus colubriformis* and susceptible and resistant sheep. Int. J. Parasitol. 26, 1279-1286.

Wallace DS, Bairden K, Duncan JL, Fishwick G, Gill M, Holmes PH, et al. 1995. Influence of supplementation with dietary soyabean meal on resistance to haemonchosis in Hampshire down lambs. Res Vet Sci. 58:232-7.

Wallace DS, Bairden K, Duncan JL, et al. 1998.The influence of dietary supplementation with urea on resilience and resistance to infection with Haemonchus contortus. *Parasitology.* 116:67-72.

Waller PJ. 1999. International approaches to the concept of integrated control of nematode parasites of livestock. Int. J. Parasitol. 29; 155-164.

Waller, PJ., Ljungström, B-L., Schwan, O., Rudby Martin, L., Morrison, DA., Rydzik, A. 2006. Biological Control of Sheep Parasites using *Duddingtonia flagrans*: Trials on Commercial Farms in Sweden. Acta Scand., 47; 23-32.

Wang L, Wang X, Bi K, Sun X, Yang J, et al. (2016) Oral Vaccination with Attenuated *Salmonella typhimurium*-Delivered TsPmy DNA Vaccine Elicits Protective Immunity against *Trichinella spiralis* in BALB/c Mice. PLoS Negl Trop Dis 10: e0004952.

Wells P.W., Emery D.L., Hinson C.A., Morrison W.I. Murray, M. 1982. Immunization of cattle with a variant specific surface antigen of *Trypanosoma brucei*: influence of different adjuvants. Inf. Immun. 36: 1-10.

Wilbers RHP, Westerhof LB, van Noort K, Obieglo K, Driessen NN et al. 2017. Production and glyco-engineering of immunomodulatory helminth glycoproteins in plants. Scientific Reports 7:45910.

Williams, AR., Palmer, DG., Williams, IW., et al., 2010. Relationships between immune indicators of parasitic gastroenteritis, nematode burdens and faecal dry matter in sheep. Animal Production Science 50: 219-227.

Williams AR, Palmer DG. 2012. Interactions between gastrointestinal nematode parasites and diarrhoea in sheep: Pathogenesis and control. The Vet. J.192:279-285.

Windon, R.G. 1996. Genetic control of resistance to helminths in sheep. Vet.Immunol. Immunopathol. 54, 245-254.

Winter, M.D. et al. 2000. Vaccination of young lambs against infection with *Nematodirus battus* using gamma irradiated larvae. Int. J. Parasitol. 30: 1173–1176.

Witola WH, Cooks-Fagbodun S, Ordonez AR, Matthews K, Abugri DA, McHugh M. 2016. Knockdown of phosphoethanolamine transmethylation enzymes decreases viability of *Haemonchus contortus.* Veterinary Parasitology 223, 1-6.

Woodgate RG, le Feuvre A, van Burgel A, Love S, Carmichael I, et al. 2006. Experiences with WormBoss – will Australian producers make use of the internet to assist sheep worm control ? Proc. Aust. Sheep Veterinarians Ann. Conf.16: 53-60

Woodgate R, Johnsson G, Besier B. 2011. Sheep Worm Control in a Mediterranean Environment – 2011 and beyond. *Proc Aust.Sheep Vets conference*, Barossa Valley Insert, pp 8 – 11.

Woolaston, R.R., Piper, L.R., 1996. Selection of Merino sheep for resistance to *Haemonchus contortus*: genetic variation. Animal Sci. 62, 451-460.

Woolaston, R.R., Windon, R.G., 2001. Selection of sheep for response to *Trichostrongylus colubriformis* larvae: genetic parameters. Animal Sci. 73, 41-48.

Yang Y, Zhang Z, Yang J, Chen X, Cui S, et al. (2010) Oral vaccination with Ts87 DNA vaccine delivered by attenuated *Salmonella typhimurium* elicits a protective immune response against *Trichinella spiralis* larval challenge. Vaccine 28: 2735-2742.

Yang YYM, Wilson RA, Thomas SRL, Kariuki TM, van Diepen A, et al. 2018).Micro array-assisted analysis of anti-schistosome glycan antibodies elicited by protective vaccination with irradiated cercariae. J Infect Dis.

Yanming, S., Ruofeng, Y., Muleke, C.I., Guangwei, Z., Lixin, X., Xiangrui, L. (2007) Vaccination of goats with recombinant galectin antigen induces partial protection against Haemonchus contortus infection. Parasite Immunology 29, 319-26.

Yazwinski TA, Goode L, Moncol DJ, Morgan GW, Linnerud AC. *Haemonchus contortus* resistance in straightbred and crossbred Barbados Blackbelly sheep. J Anim Sci. 1981;51:279-84.

Yong WK, Saunders MJ, Morton RE, Hermon M, Mcgillivery DJ, Hooper IJ, et al. Immunology of *Ostertagia* resistance. In: Gray GD, Woolaston RR, editors. “Breeding for Disease Resistance in Sheep”. Melbourne: Australian Wool Corporation; 1991. p. 87-93.

Young RR. 1983. Populations of free-living stages of *Ostertagia ostertagi* and *O. circumcincta* in a winter rainfall region. Aust. J. Agric. Res. 34: 569-581.

Zawadzki JL, Kotze AC, Fritz JA, Johnson NM, Hemsworth JE, Hines BM, Behm CA. 2012. Silencing of essential genes by RNA interference in *Haemonchus contortus*. Parasitology 139:613-629.

Zhao D, Han K, Zhang L, Wang H, Tian Y, Huang X, Liu Q, Yang J, Liu Y, Li Y. 2018. Identification and immunogenic evaluation of T cell epitopes based on tembusu virus envelope protein in ducks. Virus Res. 257:74-81. doi:10.1016/j.virusres.2018.09.008