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Final report

Vaccination – ett möjligt alternativ till avmaskning av häst?

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Part 1: Detailed summary

Är det möjligt att skapa ett vaccin som skyddar mot infektion med hästens stora blodmask *Strongylus vulgaris*? Frågeställningen är stor för ett tre-årsprojekt men det är angeläget för hela hästsektorn att söka nya vägar för att minska risken för resistensutveckling mot antiparasitära medel. Vaccination skulle vara ett utmärkt komplement till de skötselåtgärder som tillämpas idag, speciellt som en ökning i förekomsten av *S. vulgaris* har rapporterats.

Under infektionscykeln genomgår *S. vulgaris* en rad utvecklingstadier och det är först i L4/L5 stadiet när parasiten penetrerat tarmens mucosa och nått blodbanorna som den orsakar de stora, inte sällan dödliga, skadorna. Projektets främsta syften har därför varit att:

- etablera metoder för att i laboratoriet kunna odla fram definierade larvstadier av *S. vulgaris* som efter rening och attenuering kan utvärderas som vaccinantigen
- kartlägga den immunmodulerande förmågan hos olika larvstadier av *S.vulgaris*
- utvärdera ett nytt adjuvans ”G3” i häst, speciellt med avseende på adjuvansets förmåga att påverka immunreaktionen mot parasitantigen
- utveckla en metod för att odla organoider från hästtarm

Dessa verktyg tillsammans med vår befintliga kompetens och utrustning för att studera immunreaktioner i häst lägger den väsentliga grunden för att verkligen kunna konstruera det önskade vaccinet.

Metodutvecklingen har varit lyckosam och resultat från den immunologiska karaktäriseringen av parasitantigen och adjuvanset är publicerade i *Veterinary Research* (2018) samt in press i *Parasite Immunology*. Även arbetet med att etablera organoider från hästtarm är framgångsrikt och den slutliga karaktäriseringen av organoiderna pågår. Projektet har väckt internationellt intresse och vi har idag etablerat samarbete med Glück Equine Research Center, University of Kentucky, där det för parasitforskning finns en helt unik besättning med hästar etablerad sedan 40 år tillbaka. Vi har även genom detta samarbete fått tillgång till ett rekombinant protein från *S. vulgaris* (SvSXP) som kan utgöra en vaccinkandidat. Dessutom har vi erbjudits möjligheten att utföra vaccinationsstudier på unga hästar i besättningen där de kommer att utsättas för en naturlig challenge under fältmässiga men kontrollerade former.

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Part 2: Main report

Introduction

The equine large blood worm (*Strongylus vulgaris*) is considered the most pathogenic parasite of horses. Through the years, prevention of *S. vulgaris* infection has primarily relied on regular anthelmintic treatments. However, concerns about antimicrobial resistance along with worrying reports of an increased prevalence of *S. vulgaris* (Tydén et al., 2019) motivates development of new prophylactic methods to control this parasite. This project therefore aimed to explore the feasibility of developing a vaccine against *S. vulgaris*.

Horses are infected by third stage *S. vulgaris* larvae (L3) while grazing on contaminated pasture. On pasture, the L3s are covered by a protective (L2) cuticle that is later shed off when the larvae reache the horse's gastro-intestinal tract. In the small intestine, these "exsheated L3s" penetrate the intestinal wall and invade the intestinal submucosa. There, the exsheated L3s undergo another molt, to the L4 stage. Within one or two days, the L4s start to migrate through the small blood vessels, against the blood flow, until they reach the cranial mesenteric artery. There, the larvae continue to grow for 4-5 months, molt into the fifth stage and eventually migrate back to the intestine where they sexually mature and start to produce eggs.

The pathology of *S. vulgaris* relates to the migratory phases in the parasite's life cycle. Larval migrations of L4, and later L5, in the blood vessels surrounding the cranial mesenteric artery can cause lesions, thickening of the arterial wall, clot formation and tissue necrosis (McCraw & Slocombe, 1976) which potentially can end up with life-threatening non-strangulating intestinal infarction (Phil et al., 2018). The ultimate goal of an *S. vulgaris* vaccine would consequently be to induce an immune response that could stop the infective stage (late L3-early L4) larvae from penetrating the intestinal wall and submucosa, before it can cause damage to the horse´s blood vessels.

Development of parasite vaccines has always been a great challenge. The parasite's migration through different tissues while constantly shifting between life cycle stages and capacity to modulate the immune response to its own advantage are things that need to be addressed when attempting to formulate a vaccine. Knowledge about how the immune system reacts to different life cycle stages of the parasite is therefore of great importance when selecting the antigens to include in a vaccine formula. In the present project, we addressed this issue by establishing methods to generate different larval stages of *S. vulgaris* and map immune reactions to these *in vitro*.

In order to map immune reactions a panel of markers is required. With increasing insight from our *in vitro* studies on the morphological changes during *S. vulgaris* larval development we expanded our toolbox to meet the needs for characterization of equine immune reactions to various stages of the parasite. The versatility in immune response elicited by the larval stages confirmed that an adjuvant is needed to direct the immune response appropriately. Adjuvants are immune enhancing substances that are included in most vaccines to enhance and/or modulate the immune response in a desired direction. Within this project, a novel adjuvant formulation "G3" was evaluated for its immunomodulatory properties in the horse, alone and in combination with other immunostimulatory compounds or L3 larvae.

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The immune reaction to the various stimuli used in the present project has been monitored as cytokine profiles. An early immune reaction is initiated when a foreign substance (antigen) interacts with host cells (epithelial cells, mucosal cells, sentinel immune cells) and thereby induces production of cytokines. These soluble factors will affect cells of the immune system and orchestra the ensuing specific immune response involving T helper (Th) cells. The Th cells will turn into effector subpopulations (Th1, Th2, Th17) or become down regulating T regulatory (Treg) cells dependent on the early cytokine milieu. Thus, the type of immune response can be predicted from the cytokine profile and importantly there is a possibility to (re-)direct the immune response in a desired manner by using immune modulatory compounds such as adjuvants. In nature, many microorganisms and in particular parasites are known to modulate the immune response to their own favor, allowing a productive infection in spite of the immune reaction. Therefore, it is important to determine the cytokine profile(s) induced by the parasite and to predict what kind of immune response that would hinder establishment of the infections without causing immunopathology.

Our studies of immune reactivity were first carried out using blood mononuclear cells that is the most convenient material for assessment of systemic immune reactions. However, the intestinal immune response is programmed to tolerate feed antigens as well as the normal microbiota in order to limit inflammatory reactions. To increase our understanding how the infective stage of *S. vulgaris* interacts with intestinal tissue and to assess ensuing inflammatory/immune activating signals, we have set up a 3D cell culture system, so called organoids, derived from stem cells isolated from equine small intestine. This culture system has the advantage that it mimics the composition and architecture of the intestinal epithelia, thus making it better at representing the environment *in vivo* compared to conventional two-dimensional cell line cultures. The use of organoids in research has a wide range of applications and has successfully been used to study developmental diseases, drug efficacy/toxicity, regenerative medicine and used as infection models. Due to its organ similarity, intestinal organoids are considered to be an appropriate model to study host-parasite interactions *in vitro* (Duque-Correa et al., 2020). Most organoid research has been performed using stem cells isolated from mice or man. As yet, the number of publications on livestock organoids are scarce, and there is only one article describing the procedure in equines (Stewart et al., 2018).

In order to move from *in vitro* to *in vivo* studies contact was established with DVM Martin Nielsen at Glück Equine Research Center, University of Kentucky. His group is world leading in research on *S. vulgaris* and he is nowadays in charge of two unique research herds at University of Kentucky that were initiated 40 years ago. Horses in one of these herds, referred to as the "Historic Parasitology Research Herds" (https://horseparasites.ca.uky.edu) are not dewormed and are frequently used to evaluate the impact of *S. vulgaris* on equine health and in the development of diagnostic tools. In that context, a Strongylus protein (SvSXP) has been cloned that also is a putative vaccine antigen (Andersen et al., 2013). In collaborative studies we now have access to SvSXP and have initiated *in vitro* studies with SvSXP. Furthermore, we are offered to conduct an experimental vaccination trial on young horses from the "historical herd", using SvSXP in combination with the G3 adjuvant.

Material and methods

Most of the Material and Methods are detailed in two scientific publications emerging from the project: (Hellman et al. 2018 The adjuvant G3 promotes a Th1 polarizing innate immune response in equine PBMC. Vet Res. 49:108 and Hellman et al. Cytokine responses to various larval stages of equine strongyles and modulatory effects of the novel adjuvant G3 in equine

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PBMC. Parasite Immunol. 2020 Sep 24;e12794). Below is a brief summary of methods used in these two publications and a more detailed description of the yet unpublished organoid technology.

Isolation of and *in vitro* culture of equine PBMC

Equine peripheral blood mononuclear cells (eqPBMC) were isolated by gradient centrifugation on Ficoll-Paque, washed and their viability determined before suspension in growth medium and *in vitro* culture. Blood was collected from horses belonging to the Dept of Clinical Sciences or from private owned horses (Ethical permission no. 5.8.18-08784). At *in vitro* culture, eqPBMC were exposed to various concentrations of the G3 adjuvant, synthetic compounds mimicking microbial components (FliC, Pam3CSK4), defined larval stage preparations or combinations thereof. EqPBMC grown in plain growth medium were always included as controls.

Cytokine profiling

The immune reactions have been monitored as cytokine profiles. In most cases these were determined as gene expression in stimulated cultures relative to that in the control cultures after normalization to reference genes. RT-PCR was used to quantitate the mRNA transcription which is expressed as Fold Changes (FC-values). During the course of the present project a substantial panel of qPCR assays have been optimized and validated for equine cytokines. In some cases, also ELISAs for equine IL-10 and IFN-γ, respectively have confirmed the production of these cytokines. The cells producing IFN-γ at various stimuli are currently characterized phenotypically by multicolor flow cytometry.

The G3 adjuvant

The adjuvant G3 (NanoQuilR Research Reagent; CRODA Denmark A/S) is a nanoparticle adjuvant. Its ancestor, the ISCOM Matrix, is a well-tolerated adjuvant that has been on the market for decades. For instance equine vaccines against influenza (Equip® F vet. Orion Pharma Animal Health) or combined influenza tetanus vaccines (Equip® FT vet.) are adjuvanted with ISCOM Matrix. These vaccines are known for their induction of a long-lasting immunity with a balanced Th1/Th2 response. Thus, the G3 adjuvant is expected to cause no severe side effects and influenza vaccination trials in mice confirm that also G3 has the capacity to induce both Th1 and Th2 cytokines (van de Sandt et al., 2014; Hjertner et al., 2018). The cytokine profiles induced by G3 in eqPBMC were scrutinized *in vitro* as outlined above. Also, the capacity of G3 to affect the cytokine response induced by synthetic microbial mimics or by different parasite larval stages was assessed using eqPBMC.

Parasite material

One hurdle to overcome when working with parasites is that they are rarely reproducible in the lab, as is also the case with *S. vulgaris*. A challenge related to *S. vulgaris* in particular is the long prepatent period. It takes approximately six months from initial infection until *S. vulgaris* has completed its life cycle and starts to produce eggs. As most horses in Sweden get infected on summer pasture, our best chance of collecting *S. vulgaris* eggs was at spring. Feaces was collected from horses with known *S. vulgaris* and cyathostomin infection during spring 2018, 2019 and 2020. In 2018, horses were found through advertisement on social media and SLU platforms. Later (2019 and 2020), feaces was obtained via collaboration with Eva Tydén. The parasite eggs were hatched and cultured to L3 stage using the inverted-petri dish method (van Wyk and Mayhew 2013). As *S. vulgaris* almost always occur in co-infection with cyathostomins, *S. vulgaris* L3 were isolated by collecting larvae under the microscope using a 10 µl pipette.

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Larval cultures

To obtain later stages that would correspond better to the infective stages of *S. vulgaris* we developed protocols to exsheat L3 larvae from their L2 cuticle and induce moulting to the L4 stage (Hellman et al, 2020). Briefly, the optimal conditions for exsheatment of L3 stage larvae in sodium hypochlorite were determined and the exsheated L3 larvae were thereafter used either in cell culture experiments or put into larval cultures. The larval cultures were executed using cKW2 medium that triggers moulting of *S. vulgaris* to the L4 stage (Chapman et al, 1994). After 5 days incubation in cKW2 medium shedding of the L3 cuticles was observed and the larvae had, by definition (Chapman et al., 1994), moulted to L4. These larval preparations (L3, exsheated L3 and L4) were used in cytokine profiling experiments that enabled us to map the immune response to different larval stages of importance during the early phase of infection.

Set-up and characterization of intestinal organoids and organoid derived monolayers

Intestinal organoids were set up using crypts isolated from equine mid-jejunum collected within one hour after euthanization. The procedures for crypt isolation and plating in a 3D matrix were performed according to Stieler Stewart et al. (2018) with some slight modifications. Briefly, 1-2 cm pieces of jejunum were incubated in 50 mL tubes containing PBS with EDTA, DTT and antibiotics/antifungals on a shaker, first on ice and thereafter in 37°C followed by repeated vigorous shaking. After removing the villi approximately 25-40 crypts were plated in a 3D-growth supporting cell culture medium (Matrigel basement membrane) supplemented with necessary growth factors. After one week, the organoids were fully grown and could be passaged by fragmentation of the organoids into new "crypt units". To get access to the luminal surface of the epithelia, single cells were isolated from fully grown organoids and reseeded as transwell monolayers using a modified protocol originally developed for porcine organoids (Van der Hee, 2018). The organoids and transwell monolayers was characterized on mRNA level using RT-PCR. Primers for specific cell lineage markers to identify stem cells, proliferative cells, epithelial cells, enteroendocrine cells, goblet cells and tuft cells were optimized for the assay specific primer concentration and annealing temperature and thereafter run on organoid and monolayer material.

Results and discussion

Methods were established to grow and moult various larval stages of *S. vulgaris* (L3, "exsheated" L3 and L4). In this process, necessary steps for eliminating contaminants from the faecal samples and conditions for UV attenuation of the various larval stages were elaborated. The various larval preparations were used for intial screening and comparison of their capacity to induce a cytokine response in eqPBMC.

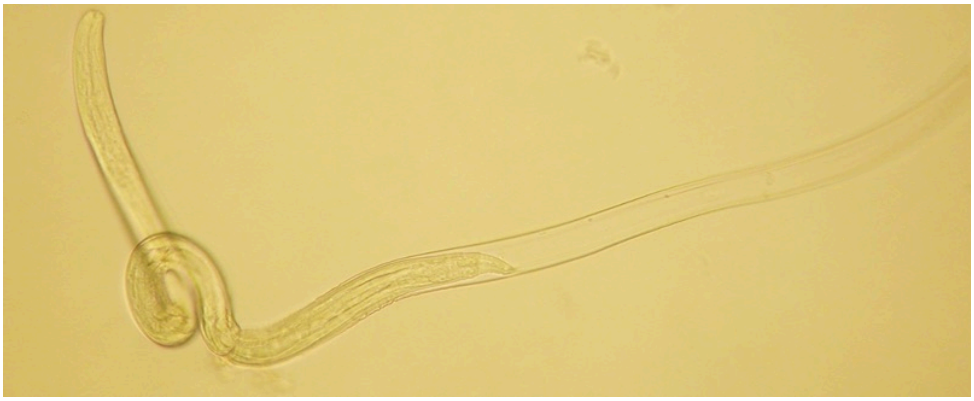

Figure 1. S. vulgaris moulting to L4 after five days of culture. Note that the L3 larvae is "exsheating", shedding its L3 cuticle and thereby changing its immunomodulatory capacity.

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S. vulgaris infective stage larvae

The outcome of an immune reaction is determined by the first response of the host to the insult, i.e., structures on or secreted compounds from the microorganism and organelles from damaged host tissue. This response can be categorized by determing early cytokine profiles and is commonly referred to as type of T helper (Th) resonse.

Our studies on the immune reaction to L3, exsheated L3 and L4 stage larvae in eqPBMC (Figure 2) show that the immune response to these different larval stages of *S. vulgaris* is diverse. The cytokine profile induced by L3 stage larvae is characterized by expression of Th2 polarizing cytokines, most prominent by IL-4. This expression was consistently high in all three larval preparations. However, the gene encoding IL-5 and IL-9 increased significantly in eqPBMC exposed to exsheated L3s. The expression of IL-5 and in particular IL-9 increased even further in the L4 exposed cultures. Although the most unexpected finding was the induction of the Th1 polarizing cytokine IFN-γ by the L4s.

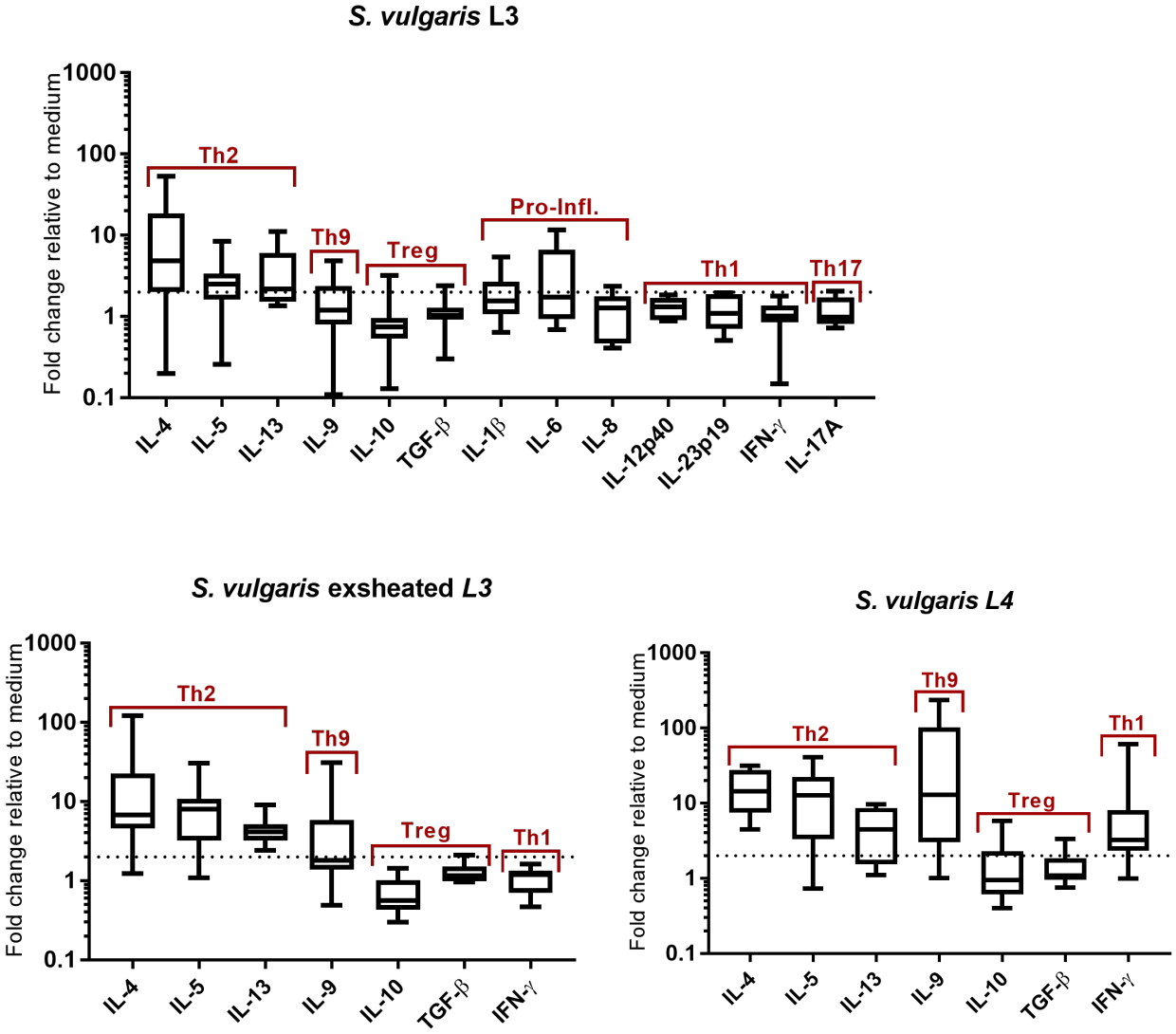

Figure 2. Relative gene expression of cytokine genes in cultures of eqPBMC exposed to the L3 stage (A), exsheated L3 (B) and L4 stage (C) of S. vulgaris.

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The G3 adjuvant

The cytokine profile induced by the G3 adjuvant in eqPBMC was dominated by Th1 polarizing cytokines (IL-12p40, IL-23p19 and IFN-γ) as well as the pro-inflammatory cytokines (IL-1β, IL-6 and IL-8) as shown in Figure 3. When G3 was combined with synthetic mimics of microbial products, the TLR5 agonist FliC or the TLR2 agonist Pam3CSK4, this profile was enhanced. In particular, a synergy effect on the production of IFN-γ was observed when G3 and Pam3CSK4 were combined. Similarly, a significant increase of IL-8 was seen when combining G3 and FliC. This suggest that the G3 adjuvant is versatile, making it possible to shape the immune reaction in a desired direction by slight adjustments of the adjuvant formula.

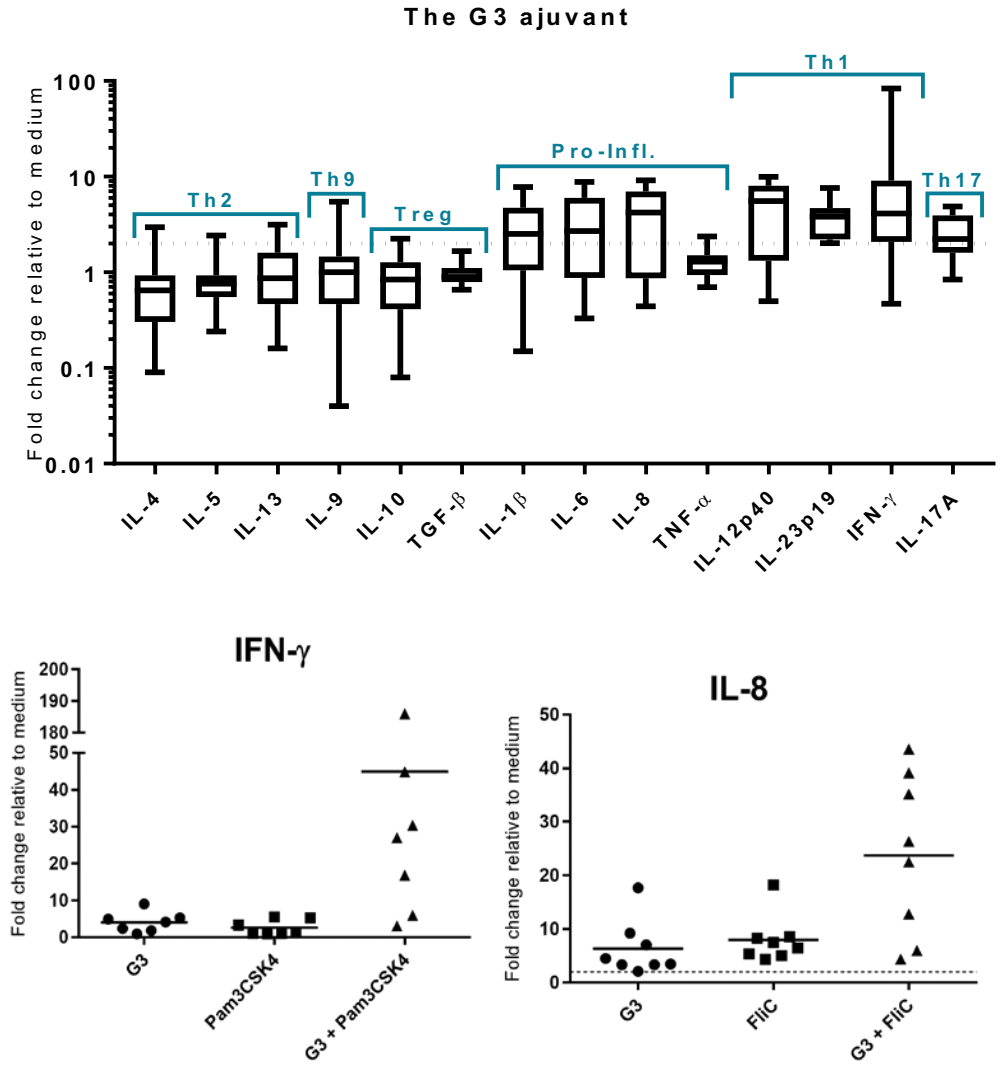

Figure 3. Relative gene expression of cytokine genes in cultures of eqPBMC exposed to the G3 adjuvant for 18 h (A) and the gene expression of IFN-γ and IL-8 after 18 h of exposure to G3 and Pam3CSK4 or G3 and FliC, respectively, alone or in a combination thereof.

The adjuvant G3 can over-ride the parasite induced immune reaction

The capacity of the adjuvant G3 to enhance the cytokine production induced by the synthetic TLR agonists was further tested *in vitro* with the L3 stage of *S. vulgaris* using eqPBMC

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(Figure 4). Interestingly, the adjuvant retained its ability to induce a Th1 type of response (IFN-γ) in the presence of L3. Furthermore, the inbuilt capacity of L3 to induce the cytokine IL-4 that directs to a Th2 response was over-ridden by the G3. Thus, the adjuvant appears to have the capacity to modulate the immune response also in the presence of parasite antigens.

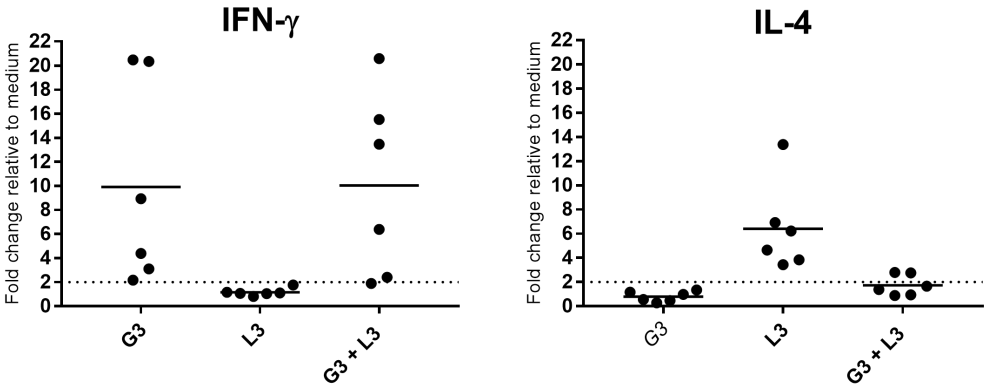

Figure 4. Relative gene expression of IFN-γ and IL-4, respectively in cultures of eqPBMC exposed to S. vulgaris L3 or the adjuvant G3 alone and combined.

Recombinant protein SvSXP from *S. vulgaris*

The early attemts to produce vaccines against *S. vulgaris* (Swiderski et al., 1999) used irradiated laral preparations as antigen. These vaccines gave partial protection but were not further progressed because of the development of easily administrated anthelminthics in the form of oral pastas. As mentioned, the growth of parasites is laborious and not a likely way to produce reproducible batches of vaccine antigen. Since then the recombinant technology for large scale production of selected antigens in bacteria has been standardized and is commonly applied for production of vaccine antigens. Recombinant antigen produced in bacteria might however have a slightly altered conformation and lack mammalian glycosylation which might affect the immune response. Data from our preliminary experiments exposing eqPBMC to the recombinant protein SvSXP are encouraging as they show a cytokine profile resembling that induced by the L4 stage of *S. vulgaris* (Figure 5). These results strengthen the relevance of using SvSXP as a candidate vaccine antigen.

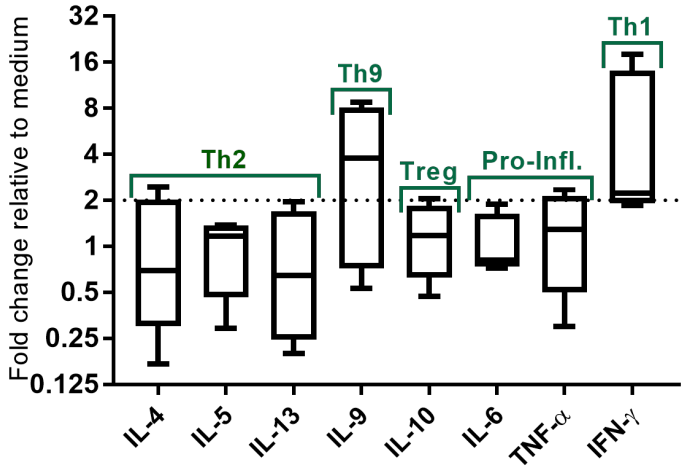

Figure 5. Relative gene expression of cytokine genes in cultures of eqPBMC exposed to the recombinant SvSXP antigen from S. vulgaris.

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Set up and culture of intestinal organoids

Crypts were successfully isolated and started to form a lumen and bud structures after 3-4 days in culture (Figure 6a). After 7-10 days, the organoids had developed more complex structures with a lumen enclosed by a visible epithelial layer and crypt-like buds (Figure 6b). In the intestine, the epithelial layer is constantly renewed and the cells at the tip of the villi go through apoptosis and are shed of. This also occur in intestinal organoids, resulting in accumulation of apoptotic cells in the lumen. In the cultures, this could be seen as accumulating darkness in the lumen at day 9-12 of culture, indicating that this was the optimal time to passage the organoids. The organoids were successfully passaged by fragmenting 7-9 days old organoids into new crypt-like structures that were plated into new matrigel domes. Both crypts, fragmented organoids and intact organoids could be frozen at -80°C and brought up to culture with more than 50 % plating efficiency (number of organoids developed from number of crypts/fragments).

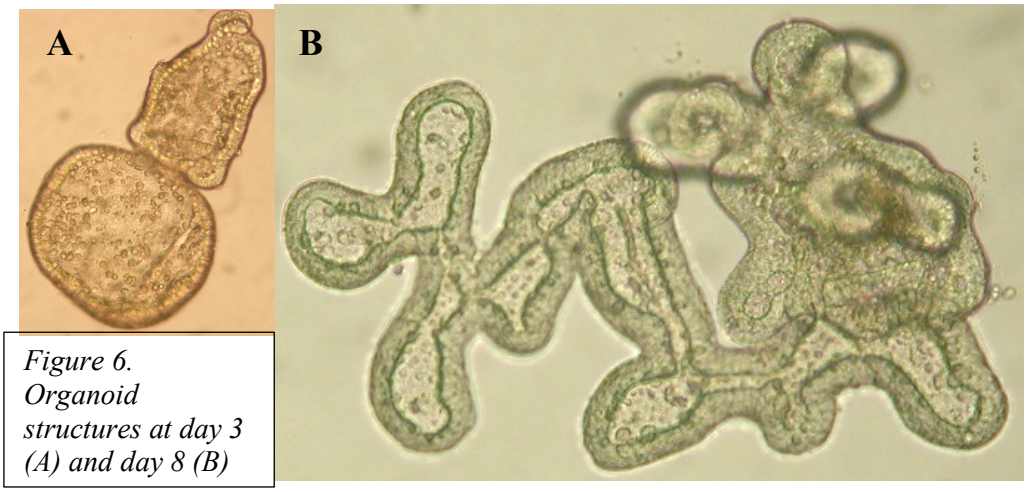

Figure 6. Organoid structures at day 3 (A) and day 8 (B)

Monolayer culture

To enable exposure experiments with parasite antigen, with or without adjuvant, easier access to the luminal surface of the epithelia is needed. Therefore, protocols were developed to dissociate the organoids to single cells and seed them into monolayer cultures on transwell plates or in cell culture plates pre-coated with a thin layer of matrigel. The optimal seeding concentration was determined to approximately 30 000 cells per cm^2. With this concentration a confluent monolayer was obtained after 3 days of culture. The cells plated in the transwell inserts also grew fast. However, both seeding concentrations tested, 150 000 or 300 000 cells, were too high resulting in that the cells grew on top of each other. This protocol is currently under optimization.

Characterization of organoids and monolayers

The cell composition of two different batches of organoids and two transwell monolayers with different seeding densities (150 000 or 300 000 cells) were characterized using RT-PCR analysis of linage specific markers (Figure 7). The preliminary results show that all markers were detected in all samples. The low variation in Cq values between the two batches of organoids indicates that the culture system is robust and reproducible. Both organoids and transwells displayed lower Cq values (i.e. higher expression) on markers for epithelial cells (EPCAM), proliferative cells (PCNA and SOX9) and stem cells (SOX9) compared to enteroendochrine (CGA), goblet cells (MUC2) and tuft cells (DCLK1) reflecting the cellular composition of the intestine *in vivo*. In addition, the small variation in Cq values between

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organoids and trans-wells indicates that organoids can be plated and cultured as a 2D monolayer without substantial loss of the original cell population.

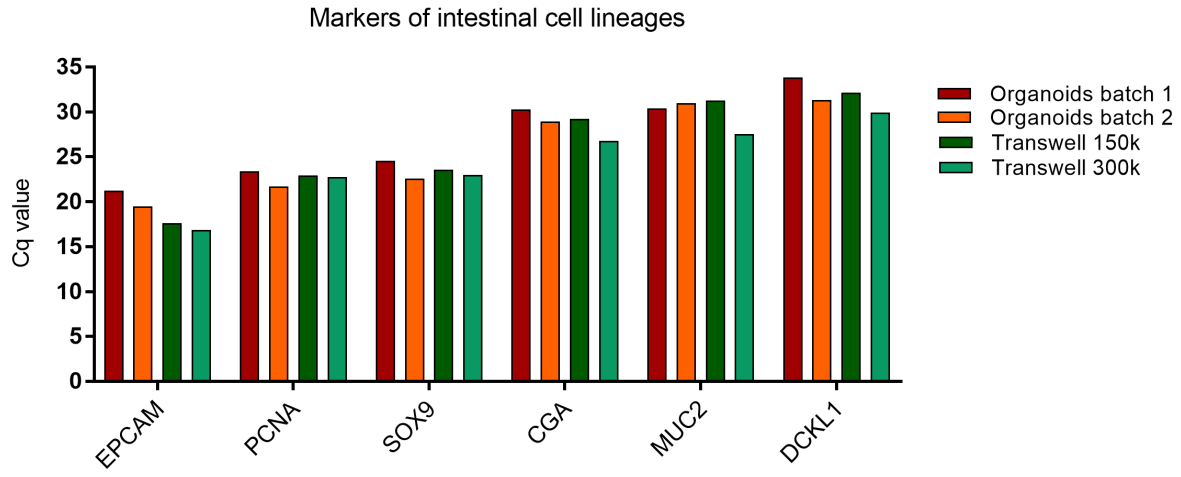

Figure 7. Expression of marker genes for different intestinal cell lineages in organoids and transwell monolayers.

Conclusions

Relevant material and methods, necessary for evaluation of immune reactions to potential *S. vulgaris* vaccine antigens have been established. This includes a capacity to estimate the relative gene expression for an assortment of equine cytokine genes. Cytokine screening of various *S. vulgaris* larval stages confirmed that the parasite indeed changes its immune modulatory capacity at moulting which has to be considered when formulating a vaccine. A candidate vaccine antigen, SvSXP, has been selected that induces a cytokine profile that is similar to that of L4 larvae. The exsheatment of cuticulae at moulting from L3 to L4 larval stage seems crucial for the invasive capacity of the parasite and appears to be accompanied by a shift in cytokine profile. In that context, the need for a versatile adjuvant is obvious and our results imply that the "G3" adjuvant fulfills these criteria for immune modulation in the horse. The analyses have so far been conducted with eqPBMC that reflects the systemic immune response. In the case of helmint infections the first encounter however is via the intestine where the immune reactivity is suppressed via regulatory T cells. Therefore, the successful achievement to grow equine intestinal organoids forms an important piece needed in the puzzle for evaluation of helmint vaccine components *in vitro*. Importantly, international collaboration is established that will enable experimental vaccination trials in horses exposed to a natural challenge in controlled facilities with exclusive access to all modern *S. vulgaris* diagnostic techniques such as qPCR and ELISA specific for SvSXP.

Relevance for the practical horse sector incl. recommendations

To reach the main objective, a vaccine against *S. vulgaris*, a continuation of the project is needed to further evaluate the SvSXP protein as a model vaccine antigen and to conduct experimental vaccinations. Experience from other microorganisms has shown us that vaccine development can take time. But considering the emergence of antimicrobial resistance with no new anthelminthics in sight, it is essential to be the frontrunner in this race. Many of the techniques for immune profiling generated within the current project can directly benefit other diciplines of equine research, such as the study of inflammatory conditions, infectious diseases, drug/treatment efficacy, biomarkers for inflammation / infection discriminating

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between viral and bacterial infections and etc. In particular, the novel techniques for establishment of intestinal organoids can be applied to many research topics and opens up for inter-disciplinary collaborations in equine research.

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Part 3: Result dissemination

State all result dissemination from the financed project into the appropriate section, including information as indicated. Additional rows can be added to the table.

Scientific publications, *published*	*Author(s), year, title, journal, Vol, No, pp. (doi/link if applicable)*
	Hellman, S., Hjertner, B., Morein, B., Fossum, C. 2018. The adjuvant G3 promotes a Th1 polarizing innate immune response in equine PBMC. Vet Res. 22:49 DOI: 10.1186/s13567-018-0602-2
	Hellman S, Tydén E, Hjertner B, Nilsfors F, Hu K, Morein B, Fossum C. Cytokine responses to various larval stages of equine strongyles and modulatory effects of the novel adjuvant G3 in equine PBMC. Parasite Immunol. 2020 Sep 24;e12794 doi: 10.1111/pim.12794
Scientific publications, *submitted*	*Author(s), title*
Scientific publications, *manuscript*	Hellman, S Early cytokine reactions to Strongylus vulgaris antigens in equine enteroid-derived epithelial monolayers
Conference publications/ presentations	*Author(s), year, title, conference name, location and date, (link if applicable)*
	Hellman, S., Tydén, E., Hjertner, B., Nilsfors, F., Morein, B., Fossum, C. 2019. Cytokine responses to infective larval stages of equine Strongyle parasites. Poster presentation at the International Veterinary Immunology Symposium (IVIS 2019), Seattle, Aug 13-16 *2019*
	Hellman, S. 2018. The adjuvant G3 promotes a Th1 polarizing innate immune response in equine PBMC. Oral presentation at the 6th European Veterinary Immunology Workshop (EVIW2018), Utrecht, Sept 5
	Hellman, S., Hjertner, B., Morein, B., Fossum, C. 2017. A novel saponin adjuvant G3 modulates cytokine responses in equine PBMC. Poster presentation at the 44th annual meeting of the Scandinavian society of immuniology (SSI2017), Stockholm, Oct 17-20
	Fossum, C., Hjertner, B., Hellman, S., Bengtsson, T., Pauli, S., Morein, B. 2016. The third generation of ISCOMs. Abstract. International Veterinary Immunology Symposium, Gold Coast, Australia, Aug 16-19
Other publications, *media etc.*	*Title, year/date, place of publication (link if applicable)*
	Genombrott i jakten på immunologins gåtor. 2020 Augusti Svensk Veterinärtidning Vol 72 No 8.

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Oral communication, *to horse sector, students etc.*	*Title, year/date, group presented to (link if applicable)*
	Hellman, S. 2019. Vaccination against Strongylus vulgaris. Is it possible? Poster presentation. Hippocampus day 2019, SLU, Uppsala, Oct 30
	Hellman, S. 2018. Cytokine responses to the G3 adjuvant, TLR-agonists and bloodworm larvae in cultures of equine PBMC. Oral presentation. Equine researcher’s meeting, SLU Uppsala, Nov 18, 2018
Student theses	*Author/Student, co-authors/supervisors, year, title, type of thesis (doi/link if applicable)*
	Nilsfors, Frida, 2018. Skyddande immunitet mot Strongylus vulgaris – en framtida möjlighet? : en studie av adjuvanset G3 samt L3 larvers inverkan på cytokinproduktion hos häst. Examensarbete Veterinärprogrammet: SLU, Dept. of Biomedical Sciences and Veterinary Public Health
Other	Stina Hellman was awarded the AAI young investigator award granted by the American Association of Immunologists at the International Veterinary Immunology Symposium, August 13-16, 2019.

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