

Research Article

Cite this article: Koehler S, Springer A, Issel N, Klinger S, Wendt M, Breves G, Strube C (2023). Effects of adult *Ascaris suum* and their antigens (total and trans-cuticular excretory–secretory antigen, cuticular somatic antigen) on intestinal nutrient transport *in vivo*. *Parasitology* **150**, 78–87. <https://doi.org/10.1017/S0031182022001512>

Received: 8 June 2022
Revised: 20 September 2022
Accepted: 14 October 2022
First published online: 24 October 2022

Key words:

Ascariasis; ascariasis; ES antigen; glucose transport; GLUT2; malnutrition; roundworm; SGLT1; soil-transmitted helminths

Author for correspondence:

Christina Strube,
E-mail: christina.strube@tiho-hannover.de

Effects of adult *Ascaris suum* and their antigens (total and trans-cuticular excretory–secretory antigen, cuticular somatic antigen) on intestinal nutrient transport *in vivo*

Sarina Koehler¹, Andrea Springer¹, Nicole Issel², Stefanie Klinger², Michael Wendt³, Gerhard Breves² and Christina Strube¹ 

¹Institute for Parasitology, Centre for Infection Medicine, University of Veterinary Medicine Hannover, Hanover, Germany; ²Institute for Physiology and Cell Biology, University of Veterinary Medicine Hannover, Hanover, Germany and ³Clinic for Swine, Small Ruminants, Forensic Medicine and Ambulatory Service, University of Veterinary Medicine Hannover, Hanover, Germany

Abstract

Ascaris suum constitutes a major problem in commercial pig farming worldwide. Lower weight gains in infected pigs probably result from impaired nutrient absorption. This study investigated intestinal nutrient transport in 4 groups of 6 pigs each, which were inoculated with 30 living adult *A. suum*, or antigen fractions consisting of (1) total excretory–secretory (ES) antigens of adult worms, (2) ES antigens secreted exclusively from the parasites' body surface (trans-cuticular ES) and (3) cuticular somatic antigens of *A. suum*, compared to placebo-treated controls. Three days after inoculation into the gastrointestinal tract, glucose, alanine and glycyl-L-glutamine transport was measured in the duodenum, jejunum and ileum using Ussing chambers. Transcription of relevant genes [sodium glucose cotransporter 1 (SGLT1), glucose transporter 1 (GLUT1), GLUT2, hypoxia-inducible factor 1-alpha (Hif1 α), interleukin-4 (IL-4), IL-13, signal transducer and activator of transcription 6 (STAT6), peptide transporter 1 (PepT1)] and expression of transport proteins [SGLT1, phosphorylated SGLT1, GLUT2, Na⁺/K⁺-ATPase, amino acid transporter A (ASCT1), PepT1] were studied. Although no significant functional changes were noted after exposure to adult *A. suum*, a significant downregulation of jejunal GLUT1, STAT6, Hif1 α and PepT1 transcription as well as ileal GLUT2 and PepT1 expression indicates a negative impact of infection on transport physiology. Therefore, the exposure period of 3 days may have been insufficient for functional alterations to become apparent. In contrast, *A. suum* antigens mainly induced an upregulation of transport processes and an increase in transcription of relevant genes in the duodenum and jejunum, possibly as a compensatory reaction after a transient downregulation. In the ileum, a consistent pattern of downregulation was observed in all inoculated groups, in line with the hypothesis of impaired nutrient transport.

Introduction

Despite strict hygiene measures in many countries, *Ascaris suum* remains a significant problem in commercial pig farming worldwide. The high degree of tenacity of *A. suum* eggs in the environment hampers anthelmintic treatment efforts (Mejer and Roepstorff, 2006; Dold and Holland, 2011), leading to a high prevalence and subsequent economic losses due to liver condemnations and lower feed conversion efficacy (e.g. Boes *et al.*, 2010; Thamsborg *et al.*, 2013). After oral infection, third-stage larvae migrate *via* mesenteric veins to the liver and lungs and enter the small intestine again from approximately day 10 post infection (pi) (Roepstorff *et al.*, 1997; Dold and Holland, 2011). Reaching patency after 6–8 weeks pi, females produce approximately 200 000 eggs per day on average (Sinniah, 1982). *Ascaris suum* is closely related to the human parasite *Ascaris lumbricoides*, which infects approximately 807–1121 million people worldwide (CDC, 2020), resulting in malnutrition and growth retardation especially in children (Hlaing, 1993; O'Lorcain and Holland, 2000). Furthermore, *A. suum* has been identified as an important cause of human ascariasis in endemic areas (Zhou *et al.*, 2012). Therefore and due to the high degree of relatedness between *A. lumbricoides* and *A. suum*, which may hybridize (Zhou *et al.*, 2012; Easton *et al.*, 2020) and are even regarded as the same species by some authors (Leles *et al.*, 2012), new insights into the parasite–host interaction of *A. suum* may also be transferable to human ascariasis.

Ascaris suum-related changes in porcine intestinal nutrient absorption have been investigated during the pre-patent phase of the infection, with mixed results. On the one hand, a significant decrease of electrogenic glucose transport was recorded in the porcine jejunum at day 14 after infection with 10 000 (Dawson *et al.*, 2005) and day 21 after infection with 25 000 *A. suum* eggs (Solano-Aguilar *et al.*, 2018). On the other hand, Koehler *et al.* (2021a) were not able to reproduce these observations regarding glucose transport on days 14 and 21 pi, although using a comparable infection dose of 10 000 eggs both as a single- and a

© The Author(s), 2022. Published by Cambridge University Press. This is an Open Access article, distributed under the terms of the Creative Commons Attribution-NonCommercial licence (<http://creativecommons.org/licenses/by-nc/4.0/>), which permits non-commercial re-use, distribution, and reproduction in any medium, provided the original article is properly cited. The written permission of Cambridge University Press must be obtained prior to any commercial use.

trickle-infection, and noting altered transcription patterns of several nutrient transport-related genes. Immunologically mediated parasite expulsion between days 14 and 21 pi (Roepstorff *et al.*, 1997; Masure *et al.*, 2013) may lead to considerable variation in worm burden between individual animals and may have caused these inconsistent results. However, Koehler *et al.* (2021a) observed a significant decrease in alanine and peptide transport on day 49 pi, i.e. at the end of the pre-patent phase. Furthermore, the related roundworm *Ascaridia galli* significantly impaired intestinal glucose and alanine absorption in chicken at 5 weeks pi, i.e. at the end of the pre-patent period and therefore at a time point comparable to day 49 pi for *A. suum* (Schwarz *et al.*, 2011). When excretory–secretory (ES) antigens of adult *A. suum* were added to porcine jejunal mucosa in an *in vitro* set-up, an immediate impairment of glucose and alanine transport was evident (Koehler *et al.*, 2021b). Therefore, adult parasites may exhibit a stronger modulatory effect on the porcine mucosa as compared to larval or pre-adult parasite stages; however, this has not been investigated yet *in vivo*.

Regarding the underlying mechanisms of the observed transport impairment, an increase in the transcription of the Th2-associated cytokines interleukin-4 (IL-4) and/or IL-13, as observed e.g. in *A. galli*-infected chicken (Schwarz *et al.*, 2011) as well as in previous studies on *A. suum* (Dawson *et al.*, 2005; Solano-Aguilar *et al.*, 2018), may trigger a reduction of the intestinal sodium-coupled glucose absorption by activating the signal transducer and activator of transcription 6 (STAT6) signalling pathway (Madden *et al.*, 2002; Zhao *et al.*, 2003). Consequently, infection with the nematode *Heligmosomoides polygyrus* leads to reduced intestinal glucose absorption in wild type, but not in STAT6-deficient mice (Madden *et al.*, 2004). Furthermore, in a study on *Nippostrongylus brasiliensis*-infected mice, the sodium glucose cotransporter 1 (SGLT1) showed a reduced activity and the expression of glucose transporter 2 (GLUT2) was downregulated, whereas glucose transporter 1 (GLUT1) showed a compensatory increase, presumably induced by hypoxia-inducible factor 1-alpha (Hif1 α) (Notari *et al.*, 2014).

Transport activity and localization of SGLT1 (in intracellular compartments vs the plasma membrane) can be altered directly by protein kinase A (PKA) due to phosphorylation (Subramanian *et al.*, 2009). However, in pigs, it is not yet clear whether SGLT1 phosphorylation *via* PKA results in an increased or a decreased transporter activity (Klinger and Breves, 2018; Klinger *et al.*, 2018). Additionally, leucotriene treatment of rat intestinal epithelial cells (IECs) resulted in a reduced absorption of amino acids due to phosphorylation of the neutral amino acid transporter A (ASCT1) by PKA (Arthur and Sundaram, 2014). Phosphorylation *via* PKA may also impair the activity of Na⁺/H⁺-exchanger 3 (NHE3), subsequently changing the intracellular pH, which affects the function of peptide transporter 1 (PepT1) (Thwaites *et al.*, 1999; Kennedy *et al.*, 2002).

To provide more insight into the modulation of intestinal nutrient transport processes by *A. suum*, this study aimed at characterizing changes in the functional, transcriptional and expression levels after exposure of parasite-naïve pigs to different adult *A. suum* antigen fractions. To complete the picture, 1 group of parasite-naïve pigs was exposed to adult *A. suum* worms by gastric tube transfer to include natural conditions of antigen release as well as ES and cuticular somatic antigen (CSO) interactions.

Materials and methods

Preliminary work and animals used in this study

To test whether the controlled application of adult roundworms *via* gavage into the stomach of pigs results in establishment of infection, 10 pigs and 130 viable *A. suum* specimens were used.

Each pig received 10–16 adult *A. suum*, which were flushed into the stomach through a plastic tube (1.5 cm diameter) with tap water during anaesthesia. For the following 10 days, daily coproscopical examinations [flotation using saturated sodium chloride (NaCl) solution and modified McMaster technique] were conducted and the entire fecal mass was collected and screened for expelled roundworms. By day 2 pi, all animals showed *A. suum* egg excretion, which lasted until day 10 pi in 9 pigs and until day 9 pi in 1 pig (Supplementary Table 1). During the first 3 days pi, i.e. the period relevant for the main experiment, 12 expelled worms (0–3 per animal) were noted. On day 11 pi, anthelmintic treatment was conducted, resulting in worm expulsion in 9 pigs. Only the animal which had been coproscopically negative on day 10 pi did not excrete any worms post-treatment. These findings confirmed previous transfer attempts by Jungersen *et al.* (1996), and showed that infection of pigs with adult *A. suum*, bypassing the natural larval migration, is possible.

Weaned German landrace (hybrid) fattening pigs (17 male, 19 female), approximately 10 weeks of age and weighing 30 kg, were obtained from the Ruthe Research and Education Farm of the University of Veterinary Medicine Hannover, which is free of *A. suum*, and kept at the Clinic for Swine, Small Ruminants and Forensic Medicine, University of Veterinary Medicine Hannover. For parasitological monitoring, fecal samples were taken after arrival and on the day of necropsy (days 11–48 after arrival) and analysed with the combined sedimentation–flotation method using saturated zinc sulphate solution as flotation medium. The animals were kept in groups of 8–15 individuals on straw bedding and received standard pig diet (Deuka Ferkelstarter Primo, Düsseldorf, Germany) at recommended rates and water *ad libitum*. Prior to experimental inoculations, animals were allowed to acclimatize for a minimum of 7 days.

Inoculation of pigs with adult *A. suum* worms by gastric tube transfer

For oral transfer of adult worms collected from an abattoir at the same day, 12 pigs were anaesthetized [azaperone 2 mg kg⁻¹ BW (Stresnil®, Elanco Animal Health, Bad Homburg, Germany); ketamine 20 mg kg⁻¹ BW, Ursotamin®, Serumwerk Berneburg Tiergesundheits GmbH, Bernburg, Germany] and 6 of them were inoculated with 30 living adult *A. suum* (male:female ratio, 1:2). Worms were flushed individually through a gastric tube (outer diameter 1.5 cm) with a total amount of 1.5 L tap water. The 6 control animals were given 1.5 L of tap water only. Excess water was drained through the tube in regular intervals.

After adult worm transfer, the 6 inoculated pigs were separated by grids to collect all individual feces until necropsy 3 days later. Collected feces were rinsed through a sieve and the sieve residues were examined for the presence of *A. suum* specimens.

Adult *A. suum* ES and CSO antigen production

Adult *A. suum* antigen fractions (total ES, trans-cuticular ES and CSO antigens) were produced by *in vitro* cultivation of 420 adult worms collected at a commercial pig abattoir as described previously (Koehler *et al.*, 2021a). Briefly, total ES and trans-cuticular ES were each obtained by incubating 210 worms (male:female ratio of 1:2 to reflect natural distribution) in Roswell Park Memorial Institute (RPMI) medium for 3 days at 37°C and 5% carbon dioxide. To obtain trans-cuticular ES, the worms' mouth and anal openings were sealed with tissue glue prior to incubation. ES-containing medium was collected every 24 h and replaced by fresh medium. The different antigen fractions were centrifuged, sterile-filtered and pooled before concentration by lyophilization.

CSO was produced by homogenizing the cuticles of 210 adult *A. suum* (male:female ratio, 1:2) after shock-freezing in liquid nitrogen. The resulting homogenate was dissolved in 700 mL double-distilled water.

Inoculation of pigs with adult *A. suum* antigens

To prevent gastric degradation after oral application, *A. suum* antigens were directly applied into the gut. Laparotomy of 24 pigs was performed under anaesthesia via injection as described above, with 6 pigs each receiving either total ES, trans-cuticular ES or CSO antigens *via* injection into the proximal duodenum using a 21 G cannula, while further 6 pigs served as placebo-treated controls. The applied volume per pig corresponded to the amount of ES antigens produced by 30 worms over 3 days or the CSO antigen of 30 worms, respectively, resulting in the application of 131.5 mg total ES antigen (136 mL, protein concentration: $967.1 \mu\text{g mL}^{-1}$), 279.8 mg trans-cuticular ES antigen (216 mL, protein concentration: $1295.3 \mu\text{g mL}^{-1}$) and 329.66 mg of the CSO antigen (100 mL, protein concentration: $3296.6 \mu\text{g mL}^{-1}$). The control group received 150 mL 0.9% NaCl solution.

Collection of pig intestinal mucosa samples

Pigs were euthanized 3 days after inoculation by bolt shot and subsequent exsanguination. After immediate removal of the intestines, intestinal tissue samples of approximately $5 \times 3 \text{ cm}^2$ each were taken for quantitative real-time polymerase chain reaction (qPCR) and immunoblots approximately 1 m (duodenum) and 6 m (jejunum) distal of the pylorus as well as 1 m (ileum) proximal of the ileocaecal valve. The total jejunal length was on average 12 m, thus, the position of sampling at 6 m reflects the main location of adult roundworms in the middle of the jejunum (Nejsun *et al.*, 2009). Subsequently, the mucosa was stripped with a glass slide, snap-frozen in liquid nitrogen and stored at -80°C until further use.

For Ussing chamber experiments, intestinal segments measuring 50 cm in length were taken directly distal of the aforementioned sampling sites, rinsed with ice-cold 0.9% NaCl and stored in Ussing buffer as previously described (Koehler *et al.*, 2021a, 2021b).

Functional analyses of nutrient transport by Ussing chamber experiments

After opening and removal of the serosa, intestinal tissues were mounted in Ussing chambers with an exposed mucosal surface area of 1.00 cm^2 to measure electrogenic nutrient transport (Clarke, 2009). Eight Ussing chambers were used per intestinal segment and animal, of which 4 chambers were used for glucose transport measurements and 2 chambers each for alanine and glycyl-L-glutamine (glygln) transport measurements.

The composition of Ussing buffers and the basic procedure have been previously described by Koehler *et al.* (2021a, 2021b). Briefly, after setting the potential differences to 0 mV, 20 min of equilibration were allowed before the tested substrates (either 5 mM glucose, 10 mM alanine or 10 mM glygln) were added to the mucosal side of the respective chambers and the short-circuit current (I_{sc}) as well as tissue conductance (G_t) were measured. In order to obtain glucose flux rates, $5 \mu\text{Ci } ^3\text{H-D-glucose}$ were added to the glucose-containing chambers 10 min after the addition of 5 mM glucose to either the mucosal or the serosal side. From the radioactive side, $50 \mu\text{L}$ samples were taken after 20 min of equilibration and 60 min after $5 \mu\text{Ci } ^3\text{H-D-glucose}$ addition, additionally to 4 $500\text{-}\mu\text{L}$ samples which were obtained in 15 min intervals from the unlabelled side starting 20 min after the addition of labelled glucose. Radioactivity was measured in decays per min. At the end of each experiment, $10 \mu\text{M}$ forskolin was added to serosal

side to test for tissue viability. Finally, ΔI_{sc} and ΔG_t were calculated by subtracting the basal value before substrate addition from the maximal value after addition, and the net glucose flux rate (J_{net}) was determined by subtracting the serosal-to-mucosal flux rate (J_{sm}) from the mucosal-to-serosal flux (J_{ms}).

Transcriptional qPCR analysis of nutrient transport-associated genes

RNA extraction, cDNA synthesis and qPCR experiments were conducted as described by Koehler *et al.* (2021a). In brief, mRNA extraction from 30 to $40 \mu\text{g}$ of frozen duodenal, jejunal and ileal mucosa was achieved with the GenElute Direct mRNA Miniprep Kit (Sigma-Aldrich, Taufkirchen, Germany) and the RNA to cDNA EcoDry™ Premix (OligodT) stripes (Takara Bio Europe, Frankfurt am Main, Germany) were used for cDNA synthesis. For qPCR experiments, cDNA was diluted 1:10 in RNase-free water and stored at -80°C until further use.

Differential transcription of nutrient transport-associated target genes (SGLT1, GLUT1, GLUT2, Hif1 α , IL-4, IL-13, STAT6, PepT1) in relation to 2 reference genes (TATA box binding protein and peptidyl prolyl isomerase A as recommended by Park *et al.*, 2015) was quantified by duplex qPCR, based on primer-TaqMan MGB probe combinations designed by Koehler *et al.* (2021a). To quantify transcription levels and amplification efficiencies, plasmid standard dilution series (10^0 – 10^6 copies per μL) for each gene were included in each duplex qPCR run.

Each plate included the co-detection of 2 genes of interest and the 2 reference genes, the respective serial plasmid standard dilutions and a no-template control. Each sample was analysed in duplicate, and each run was replicated ($n = 4$ technical replicates per sample). Results were analysed with qBase+ software (version 3.2, Biogazelle, Ghent, Belgium). Averaged baseline-corrected sample fluorescence (dRn) was normalized against reference genes, inter-run calibrated and corrected for run-specific amplification efficiencies to obtain the relative quantities of each target gene. Therefore, the average of all samples was set to 1 and used as calibrator.

Expression analysis by semi-quantitative immunoblotting

Immunoblot analyses were conducted on jejunal and ileal mucosa to investigate differential expression of the transporters SGLT1, phosphorylated (p)SGLT1, GLUT2, ASCT1 and PepT1. In addition, expression of Na^+/K^+ -ATPase was assessed, as activity of ASCT1 and NHE3, which may affect the function of PepT1, is dependent on available sodium.

The apical membrane (for SGLT1, pSGLT1, ASCT1 and PepT1) and the basolateral membrane (for GLUT2 and Na^+/K^+ -ATPase) of IECs were prepared from 1.5 g of intestinal mucosa by sub-cellular fractionation as previously described (Koehler *et al.*, 2021a). Briefly, tissue was homogenized and separation of apical from crude membrane proteins (containing the basolateral proteins) was achieved through precipitation by dropwise addition of calcium chloride (final concentration of 100 mM). To enrich the crude membrane proteins, 2 mL of the homogenate were centrifuged (25 830 g, 4°C , 60 min). For enrichment of apical membrane proteins, 7.5 mL of the homogenate was centrifuged at 2000 g at 4°C for 30 min, followed by a second centrifugation step of 4 mL of the resulting supernatant (25 830 g, 4°C , 60 min). The pellet was dissolved in resuspension buffer (10 mmol L^{-1} TRIS base, 150 mmol L^{-1} NaCl, pH 7.4) with the addition of Protease Inhibitor Cocktail (P8340) and Phosphatase Inhibitor Cocktail (P5726) (Sigma-Aldrich, Taufkirchen, Germany).

For immunoblotting according to Koehler *et al.* (2021a), samples were loaded on sodium-dodecyl-sulphate-polyacrylamide

gels and transferred to nitrocellulose membranes (Amersham, Freiburg, Germany). Membranes were incubated with the primary antibody at 4°C overnight, washed 3 times with TBS-Tween and then incubated with the respective horseradish-peroxidase conjugated secondary antibody for 90 min at room temperature. To measure the pSGLT1/SGLT1 ratio, the protocol was modified as follows: after incubation with the primary pSGLT1 antibody, the membrane was stripped, washed twice with TBS-Tween and blocked again. Afterwards, the membrane was exposed to the primary SGLT1-specific antibody before incubation with the respective secondary antibody. SuperSignal® West Dura Extended Duration Substrate was used for detection of SGLT1, ASCT1, PepT1 and Na⁺/K⁺-ATPase while SuperSignal® West Femto Maximum Sensitivity Substrate (both from Thermo Fisher Scientific, Waltham, USA) was used for pSGLT1 and GLUT2 according to the manufacturer's instructions. Images were taken with ChemiDoc™ MP imaging system and band intensities were analysed with Image Lab 5.2.1 software (both from Bio-Rad Laboratories, Feldkirchen, Germany).

To normalize target band intensity to the total protein content of the samples, membranes were stained with Indian ink [fountain pen ink 4001, Pelikan, Hannover, Germany, with 2% (v/v) glacial acetic acid] and the ratio between total and target protein contents was calculated.

Statistical analyses

The results of short-circuit current (ΔI_{sc}), glucose net flux rates (J_{net}), transcription and expression analyses are presented as arithmetic means \pm s.d. Comparisons between exposure and control groups were performed by Mann-Whitney *U* tests using GraphPad Prism (v 8.0.1, San Diego, California, USA). Differences were regarded as statistically significant if $P \leq 0.05$.

Results

Parasitological analyses

All fecal samples taken from the study animals after arrival were coproscopically negative for helminth eggs or other parasite

stages. On the day of necropsy, the control animals as well as the animals inoculated with the different *A. suum* antigens remained coproscopically negative, while the 6 pigs experimentally infected by adult worm transfer were positive for *A. suum* eggs. During examination of deposited feces, expulsion of 2 worms 1 day prior to necropsy was noted in 2 pigs each, while 1 pig expelled 3 worms. By necropsy, milk spots were not observed in any of the experimental animals.

Nutrient transport modulation analyses

A comprehensive overview of the results of the functional, transcriptional and expression analyses in the duodenum, jejunum and ileum after exposure of pigs to adult *A. suum* worms or total ES, trans-cuticular ES and CSO antigens is given in Fig. 1.

Functional modulations in short-circuit currents (ΔI_{sc}) and glucose net flux rates (J_{net})

The results of trans-epithelial transport processes in the different intestinal sections are displayed in Fig. 2 and Supplementary Table 2, showing that after exposure of pigs to adult *A. suum* worms or trans-cuticular ES antigens, no significant effects on nutrient transport were recorded on any functional level. By contrast, in pigs inoculated with total ES antigens, a significantly increased ΔI_{sc} of glucose ($P = 0.026$) as well as an increased net flux rate of ³H-D-glucose (J_{net} ; $P = 0.026$) were detected in the duodenum. Furthermore, the transport (ΔI_{sc}) of alanine and glyln was significantly increased in the duodenum ($P = 0.026$ and $P = 0.009$) as well as in the jejunum ($P = 0.015$ and $P = 0.015$).

Pigs exposed to CSO antigens showed a significant decrease of the ³H-D-glucose net flux rate in the ileum ($P = 0.041$), but – similar to the total ES antigen-treated pigs – an increased alanine transport in the duodenum ($P = 0.015$) and jejunum ($P = 0.002$), while glyln transport was increased in the jejunum only ($P = 0.002$).

Functional modulations in tissue conductance (G_t)

Just before the addition of the substrates glucose, alanine or glyln, G_t was compared between the exposed and control groups.

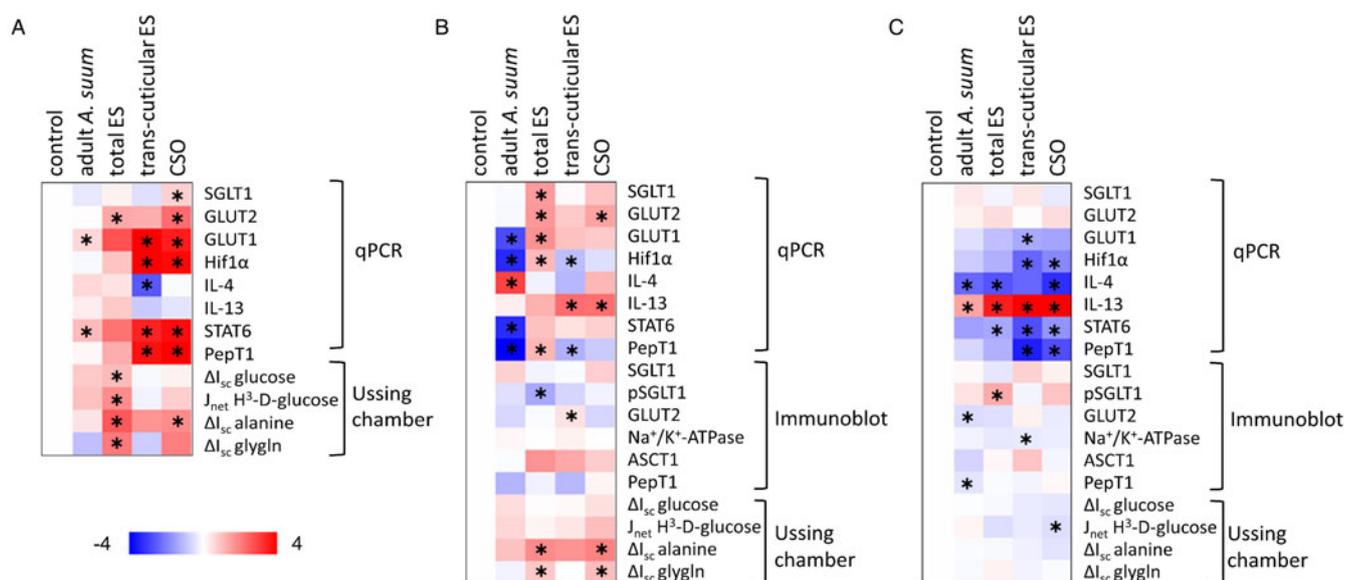


Fig. 1. Heat maps showing the decreased (blue) or increased (red) mean fold-change of transcriptional, expression and functional data of pigs exposed to adult *Ascaris suum* worms or different antigen fractions, respectively, compared to the respective control group in the duodenum (a), jejunum (b) and ileum (c). Significant differences as determined by Mann-Whitney *U* test are indicated with asterisks ($P \leq 0.05$). ES, excretory-secretory antigen; CSO, cuticular somatic antigen.

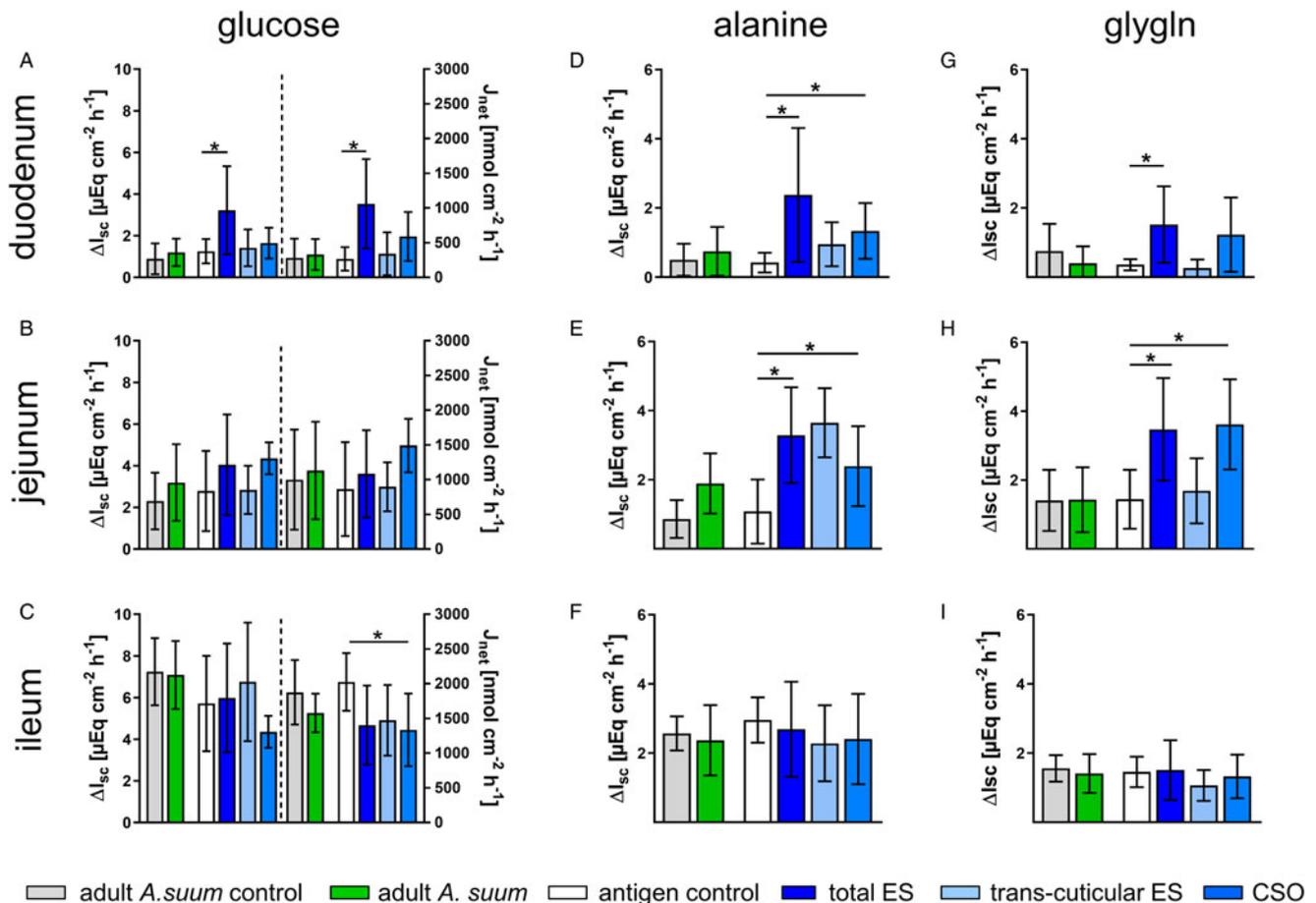


Fig. 2. Results of Ussing chamber measurements on duodenal, jejunal and ileal mucosa 3 days after exposure to adult *A. suum* worms or total ES, trans-cuticular ES and CSO antigens. Graphs display the short-circuit current (ΔI_{sc}) in response to mucosal addition of 5 mM glucose (left y-axis) and the calculated ^3H -glucose net flux rate after the addition of $5\mu\text{Ci } ^3\text{H-D-glucose}$ (J_{net} , right y-axis) (a-c) as well as short-circuit currents (ΔI_{sc}) in response to mucosal addition of 10 mM alanine (d-f) or 10 mM glyln (g-i). Results are shown as arithmetic means \pm s.d. Significant differences as determined by Mann-Whitney *U* test are indicated with asterisks ($P \leq 0.05$). ES, excretory-secretory antigen; CSO, cuticular somatic antigen.

Pigs exposed to adult *A. suum* worms showed a higher G_t in the ileum chambers used for alanine and glyln transport measurements ($U = 0$, $P = 0.002$ each). Further, G_t significantly increased in the jejunum of pigs exposed to total ES antigens in glucose ($U = 4$, $P = 0.026$) and alanine ($U = 2$, $P = 0.009$) chambers. In pigs exposed to trans-cuticular ES antigens, G_t decreased in the duodenum, used in chambers for glucose ($U = 5$, $P = 0.041$) and glyln ($U = 1$, $P = 0.004$) measurements, and in the jejunum in glyln chambers ($U = 1$, $P = 0.004$). No significant changes in G_t were noted in the ileum of any exposed group.

Transcriptional modulations of nutrient transport-associated genes

Transcriptional data of analysed genes associated with intestinal nutrient transport are presented in Fig. 3 and Supplementary Table 3. In pigs exposed to adult roundworms, the transcription rate of GLUT1 and STAT6 significantly increased in the duodenum ($P = 0.026$ each), but decreased in the jejunum ($P = 0.002$ and $P = 0.004$). Further, Hif1 α and PepT1 transcriptions decreased ($P = 0.002$ each), while IL-4 transcription was 16 times higher compared to the control group ($P = 0.002$). In the ileum, however, IL-4 transcription underwent a significant decrease ($P = 0.017$) and IL-13 was increased ($P = 0.026$, Fig. 3c).

Regarding exposure to adult *A. suum* antigens, striking differences were noted between the different antigens and intestinal sections, respectively. After total ES antigen inoculation only the

duodenal GLUT2 transcription was altered ($P = 0.041$, Fig. 3d). This increase remained in the jejunum, where additionally the 2 other glucose transporters as well as Hif1 α and PepT1 were upregulated (GLUT1 and GLUT2: $P = 0.004$; SGLT1 and Hif1 α : $P = 0.002$; PepT1: $P = 0.026$). However, none of these genes was significantly regulated in the ileum, where IL-13, IL-4 and STAT6 were significantly affected by either up- (IL-13, $P = 0.002$) or down-regulation (IL-4: $P = 0.041$; STAT6: $P = 0.015$).

Following exposure to trans-cuticular ES antigens, most genes were significantly regulated in the duodenum, with GLUT1 ($P = 0.004$), Hif1 α , STAT6 and PepT1 ($P = 0.002$ each) showing increased transcription compared to the control group, while IL-4 transcription was decreased ($P = 0.002$). In the jejunum, Hif1 α and PepT1 showed a decreased transcription ($P = 0.015$ and 0.041), while the other mentioned genes returned to unaltered levels. Additionally, jejunal transcription of IL-13 was significantly affected in terms of an upregulation ($P = 0.015$). The described significant jejunal regulations of Hif1 α , PepT1 and IL-13 persisted in the ileum ($P = 0.002$ each) in addition to decreased levels of GLUT1 and STAT6 ($P = 0.002$ each).

Exposure to CSO antigens led to the most significant effects in the duodenum, with increased transcription rates of each glucose transporter (SGLT1 and GLUT2, $P = 0.009$; GLUT1, $P = 0.026$) as well as Hif1 α ($P = 0.002$) and STAT6 ($P = 0.004$), while PepT1 transcription decreased ($P = 0.002$). Of these, only GLUT2 transcription remained increased in the jejunum ($P = 0.041$), where IL-13 also underwent transcriptional upregulation ($P = 0.009$).

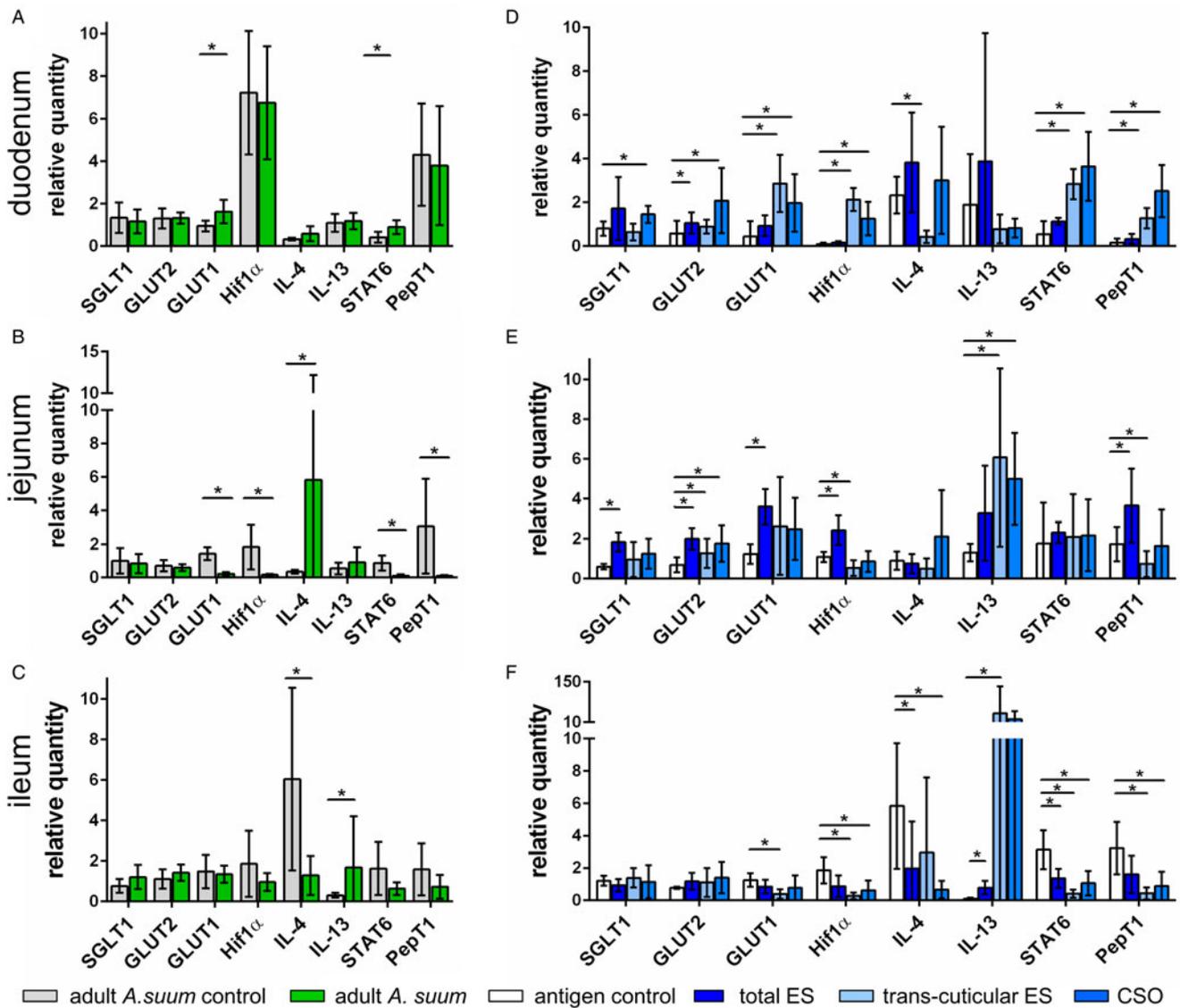


Fig. 3. Transcription data of nutrient transport-associated genes in the duodenal, jejunal and ileal mucosa 3 days after exposure to adult *A. suum* worms (a-c) or total ES, trans-cuticular ES and CSO antigens (d-f). Results are shown as arithmetic means \pm s.e. Significant differences as determined by Mann-Whitney *U* test are indicated with asterisks ($P < 0.05$). ES, excretory-secretory antigen; CSO, cuticular somatic antigen.

The latter upregulation was still observable in the ileum ($P = 0.002$), whereas the other significant transcriptional alterations in the ileum were downregulation of IL-4 ($P = 0.004$), STAT6 ($P = 0.009$), Hif1 α ($P = 0.026$) and PepT1 ($P = 0.008$).

Expressional modulations of nutrient transport-associated proteins

Nutrient transport-associated protein expression profiles examined in the jejunum and ileum are displayed in Fig. 4 and Supplementary Table 4. Pigs exposed to adult *A. suum* worms revealed no significant changes in protein expression in the jejunum, while a significant reduction of GLUT2 and PepT1 expression ($P = 0.026$ each) was noted in the ileum.

Following total ES antigen inoculation, pSGLT1 expression was decreased in the jejunum, but increased in the ileum ($P = 0.009$ each). Exposure to trans-cuticular ES antigens resulted in increased GLUT2 expression in the jejunum ($P = 0.026$), and decreased Na⁺/K⁺-ATPase expression in the ileum ($P = 0.009$). CSO antigens revealed no significant effects on protein expression, either in the jejunum or ileum. Representative immunoblots of the significantly regulated proteins are shown in Fig. 5.

Discussion

This study aimed at characterizing the effects of adult *A. suum* worms and their different antigen fractions having direct contact with the gut mucosa on small intestinal nutrient absorption. In addition to its economic importance in pig production, *A. suum* infection in pigs can be regarded as a valuable model for human ascariasis due to the close phylogenetic relationship or even conspecificity of *A. suum* with *A. lumbricoides* (Leles *et al.*, 2012; Easton *et al.*, 2020).

Based on previous *in vitro* experiments, there is strong evidence that adult worms or rather their secreted or exposed ES and CSO antigens, respectively, affect intestinal nutrient transport (Koehler *et al.*, 2021a). This study aimed to test this assumption *in vivo* by inoculating pigs with either adult *A. suum* via worm transfer, precluding previous effects on the mucosa due to *A. suum* larval development, or isolated ES and CSO antigens. As the previous *in vitro* study revealed a profound effect of total ES antigens on nutrient transport (Koehler *et al.*, 2021a), trans-cuticular ES antigen was included in this study to narrow down the antigen fractions responsible for the observed effect. Thirty adult worms per pig were chosen for inoculation as well as antigen production to mimic a realistic natural worm burden

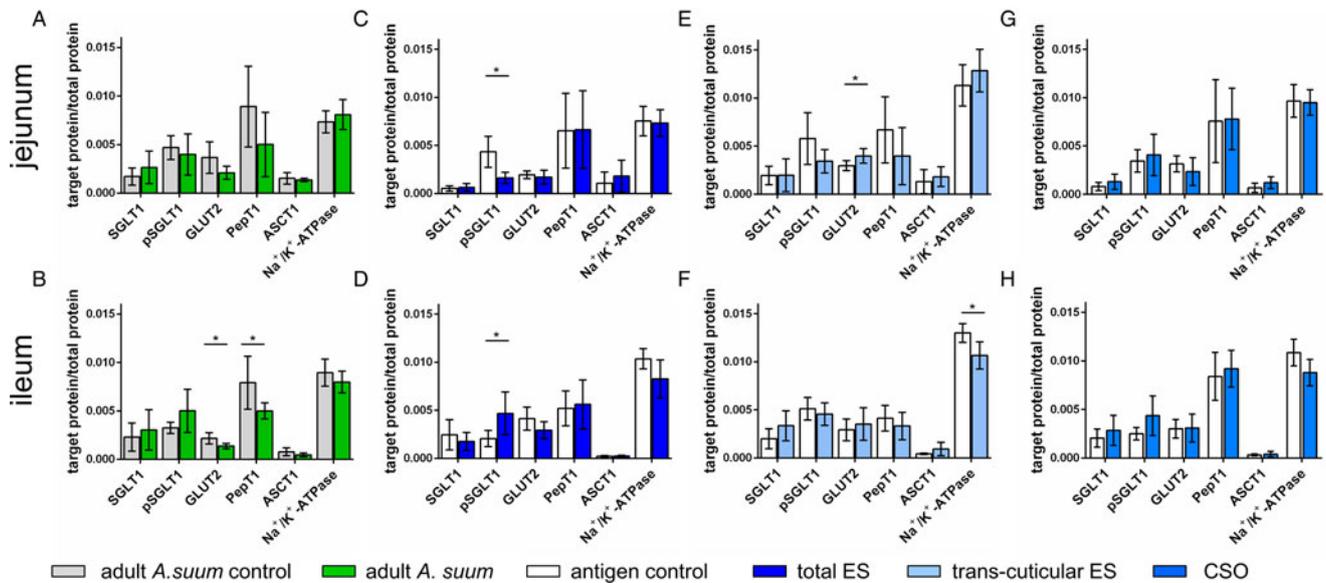


Fig. 4. Expression data of nutrient transport-associated proteins in the jejunal and ileal mucosa 3 days after exposure to adult *A. suum* worms (a, b) or total ES (c, d), trans-cuticular ES (e, f) and CSO antigens (g, h). Results are shown as arithmetic means \pm s.d. Significant differences as determined by Mann-Whitney *U* test are indicated with asterisks ($P \leq 0.05$). ES, excretory-secretory antigen; CSO, cuticular somatic antigen.

of infected pigs (Mejer and Roepstorff, 2006). A prior study on pigs experimentally infected with *A. suum* eggs did not produce any clear results regarding effects on intestinal nutrient transport, however, this may have been due to the fact that the study period encompassed mainly the pre-patent phase of the infection (days 21 and 35 pi), with only the latest investigated time point (day 49 pi) corresponding to the onset of patency and thus to the presence of mature or nearly mature worms (Koehler et al., 2021a). Here, adult worms were expected to cause a major impairment of transport processes, as they expose the mucosa to both ES and CSO antigens.

Surprisingly, however, no functional alteration of glucose, alanine nor dipeptide (glyln) transepithelial transport was measured upon exposure to adult *A. suum* worm in any intestinal segment. Nevertheless, significant transcriptional regulations of nutrient-associated genes were observed in the small intestine, which were most prominent in the jejunum. As transport processes were rapidly affected after exposure to adult *A. suum* antigens *in vitro* (Koehler et al., 2021a), the lack of a measurable functional

effect was unexpected. One explanation is that the exposure period of 3 days was not sufficient. Moreover, the antigen concentration was probably much lower than in the *in vitro* experiment (Koehler et al., 2021b), which could explain the absence of a measurable effect on the functional level. Possibly, variation in the number of successfully established worms may have also played a role. A follow-up study with a larger number of infected pigs will be necessary to investigate the quantitative effect of worm burden on alterations in nutrient transport physiology. Nevertheless, pigs also carry different worm burdens under field conditions, so a similar picture can be expected in natural situations. Despite the absence of statistically significant functional alterations, a significant downregulation of GLUT1, STAT6, Hif1 α and PepT1 transcription in the jejunum as well as GLUT2 and PepT1 expression in the ileum indicates a negative impact of infection on transport physiology. Additionally, the increased tissue conductance observed with regards to ileal mucosa used for alanine and glyln transport measurements may have masked a downregulation on the functional level in this intestinal compartment.

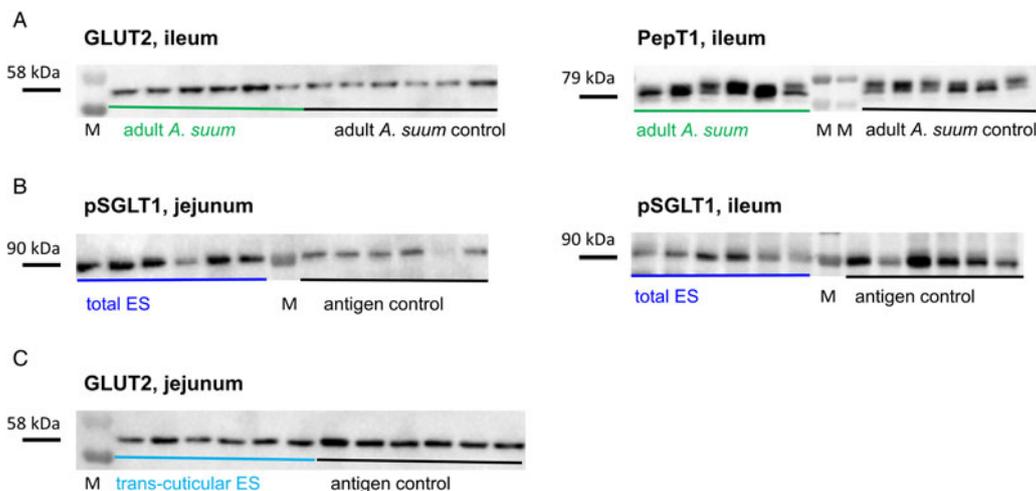


Fig. 5. Immunoblots of nutrient transport-associated proteins in the jejunal and ileal mucosa of the 6 pigs per group 3 days after exposure to adult *A. suum* worms (a) or total ES (b) and trans-cuticular ES antigens (c). Only proteins showing significant differences compared to the respective control pigs (Mann-Whitney *U* test, $P \leq 0.05$) are shown. M, molecular weight marker; ES, excretory-secretory antigen.

In contrast, GLUT1 and STAT6 transcription were increased in the duodenum. As the main location of adult roundworms is a 3 m long section in the middle of the jejunum, retrograde transport of *A. suum* antigens to the duodenum seems unlikely (Nejsum *et al.*, 2009). Rather, the increase of GLUT1 and STAT6 transcription in the duodenum may have been a compensatory reaction to the observed downregulation in the jejunum and ileum.

In contrast to the exposure to adult worms, an overall upregulation of gene transcription and an increased nutrient transport were observed in proximal parts of the small intestine after exposure to total ES and CSO antigens. In the jejunum, inoculation of these antigen fractions resulted in an increased alanine and glycin transport, whereas glucose transport was not significantly altered. However, an upregulation of all 3 glucose transporters, as well as Hif1 α and PepT1, was observed after exposure to total ES antigens, whereas pSGLT1 expression was decreased. In pigs, it is not yet clear whether SGLT1 phosphorylation *via* PKA results in an increased or a decreased transporter activity (Klinger and Breves, 2018; Klinger *et al.*, 2018). After exposure to CSO antigens, only GLUT2 transcription was significantly increased in the jejunum. Interestingly, the upregulation of transport processes was even more pronounced in the duodenum. Regarding total ES antigens, a significant increase of short-circuit currents of all nutrients (glucose, alanine and glycin) was noted in addition to increased glucose net flux rates. The enhanced transport of glucose could be due to an increase of GLUT2 expression, as the transcription rate was significantly increased. However, while exposure to CSO upregulated the transcription of all 3 glucose transporters as well as Hif1 α , no increased functional glucose transport in terms of an increased short-circuit current or net flux rate was observed. Whether this is due to a delayed translation of gene transcription to expression, e.g. in case of post-translational modifications, or to other reasons remains to be elucidated in future studies.

Trans-cuticular antigens yielded primarily upregulation in the duodenum and a mixed pattern in the jejunum, but induced no functional alterations. This might indicate that the ES fraction released *via* the mouth and/or anal opening, which is contained in the total but not trans-cuticular ES, is essentially driving the functional transport changes. It remains to be investigated whether a constant, additive or even synergistic effect is exerted on the functional level in interaction with CSO, which likewise induced increased alanine and dipeptide transport.

However, when interpreting the functional measurements, alterations of tissue conductance (G_t) should be taken into account. An increase of G_t was noted in the jejunum of the ES antigen-treated group prior to alanine addition, which may have contributed to the increase of ΔI_{sc} in these Ussing chambers. However, G_t was not altered in the duodenum, indicating that active alanine transport was indeed increased. Despite the functional increase in jejunal alanine transport, expression of ASCT1 and Na⁺/K⁺-ATPase was not significantly affected. So far, it is known that ASCT1 activity is inhibited by phosphorylation *via* protein kinase C α (PKC α) or PKA, e.g. mediated by leucotriene D4 (LTD₄) during chronic inflammation (Arthur and Sundaram, 2014). LTD₄ increases the intracellular calcium concentration, thus activating the PKC signal transduction pathway (Thodeti and Sjolander, 2002). Therefore, *A. suum* antigens might have affected the calcium channels of the IECs, which should be addressed in further studies. Likewise, dipeptide (glycin) transport was increased despite unaltered PepT1 expression. Therefore, the absorption of dipeptides may have been modulated *via* (de-)phosphorylation of the NHE3 by PKA (Zhao *et al.*, 1999; Donowitz and Li, 2007; Kocinsky *et al.*, 2007). Di- and tripeptides are transported against an

electrochemical proton gradient, which is in part maintained by NHE3 (Kennedy *et al.*, 2002; Watanabe *et al.*, 2005).

Overall, the observed increase in transport processes and gene transcription in the duodenum and jejunum was unexpected, but may have been preceded by a transient downregulation. As the antigens were applied as a single dose, the time span of 3 days until sampling may have been too long to capture such a transient effect, especially considering the small intestinal transit time of only 2–4 h (Henze *et al.*, 2021). The observed upregulation may have been a mechanism of the host to counter-regulate the preceding glucose loss or inability to absorb glucose, especially as a rather high antigen concentration was applied.

The observed upregulation dissipated further along the intestinal axis and switched to a mainly down regulatory pattern on the transcriptional level after exposure to all 3 antigen fractions in the ileum, similar to the changes induced by adult *A. suum* worms. In addition, a significantly decreased ileal glucose net flux rate (J_{net}) was measured after CSO antigen treatment. J_{net} constitutes a more accurate measurement of glucose transport than ΔI_{sc} , as ΔI_{sc} is affected by the transport of other charged molecules (Harari *et al.*, 1987; Shea-Donohue *et al.*, 2001). Overall, this confirms previous studies demonstrating reduced glucose absorption due to *A. suum* or their antigens (Dawson *et al.*, 2005; Solano-Aguilar *et al.*, 2018; Koehler *et al.*, 2021b). On reaching the ileum, the *A. suum* antigens may have already been partially degraded, resulting in a lower concentration as compared to the duodenum and jejunum, and thus causing less of a counter-regulation and more similar alterations compared to natural infections.

In the duodenum as well as in the ileum, trans-cuticular ES and CSO antigens caused similar patterns on the transcriptional level, e.g. an increase of GLUT1, Hif1 α , STAT6 and PepT1 in the duodenum, but a decrease of Hif1 α and PepT1 in the ileum. This can easily be explained by the fact that both antigen fractions probably contained similar (trans-)cuticular proteins.

Results with regard to the Th2 cytokines IL-4 and IL-13 did not fully correspond to the expected pattern. IL-13 transcription was consistently increased in all experimental groups in the ileum, but only in trans-cuticular and CSO-treated groups in the jejunum, while no significant changes were observed in the duodenum. In contrast, IL-4 transcription was increased in the jejunum, but decreased in the ileum of pigs exposed to adult *A. suum*. The decrease in the ileum was also observed after total ES and CSO treatment. It has been suggested that regulation of IL-13 primarily takes place on the receptor level, whereas regulation of IL-4 signalling occurs at the transcriptional level (Daines and Hershey, 2002). Regulation of IL-4 expression is complex and involves several transcription factors, re-stimulation by antigens and other cytokines or inflammatory molecules produced by various cell types, e.g. mast cells, and is therefore hardly predictable *in vivo* (Guo *et al.*, 2004; Paul and Zhu, 2010; Hepworth *et al.*, 2012).

Conclusions

In summary, adult *A. suum* and their different antigen fractions affected intestinal transport physiology in this study, demonstrating that larval development is not required to trigger physiological effects. However, the observed effects were not always in the expected direction and should be confirmed in future studies. For the planning of future experiments, correct exposure period seems crucial. While exposure to adult worms led to a downregulation of relevant molecules for glucose transport on the transcription and expression levels, modulation of functional transport processes was not observed within the period of 3 days. Possibly, a longer exposure time would have been needed

for functional alterations to become apparent. In contrast, 3 days after a single exposure to *A. suum* antigens may have been too late to capture a transient downregulation, and the observed upregulation may have been the result of a counter-regulatory strategy of the host in the face of the high, single-dose antigen concentration. A continuous exposure to lower antigen concentrations might be more representative of natural infections. In addition, measuring relevant metabolites in host serum will allow further insights into the metabolic consequences of altered intestinal physiology. As *A. suum* in pigs serves as a model for human ascariasis, these insights are also relevant for human health and contribute to the understanding of malnutrition in helminth-infected children.

Supplementary material. The supplementary material for this article can be found at <https://doi.org/10.1017/S0031182022001512>.

Data availability. Data supporting results are provided within the article and in the Supplementary materials.

Acknowledgements. The authors thank Ulla Küttler, Daniela Jordan, Marion Burmester, Karin Hustedt, Kerstin Kiri, Nadine Schnepel, Dirk Voigtlaender and Michael Rohde for excellent technical assistance.

Author's contributions. G. B. and C. S. conceived and planned the experiments and acquired the funding. M. W. performed sedation, surgeries and inoculation of the experimental animals. S. KO. and N. I. carried out the Ussing chamber experiments and analysed the data. S. KL. contributed to Ussing chamber data analysis. S. KO. carried out the qPCR and analysed the data. N. I. carried out the western blots and analysed the data. S. KO., A. S. and N. I. performed the statistical analyses. S. KO., A. S., S. KL., G. B. and C. S. interpreted the data. S. KO. and A. S. drafted the manuscript. S. KL., M. W., G. B. and C. S. revised and edited the manuscript. All authors read and approved the final manuscript.

Financial support. This research was funded by the Deutsche Forschungsgemeinschaft (DFG, German Research Foundation), grant numbers BR 780/23-1 and STR 1171/14-1.

Conflict of interest. The authors declare that there are no conflicts of interest.

Ethical standards. Animal experiments were performed in accordance with the German Animal Welfare act in addition to national and international guidelines for animal welfare. Experiments were permitted by the ethics commission of the Animal Care and Use Committee of the German Lower Saxony State Office for Consumer Protection and Food Safety (*Niedersächsisches Landesamt für Verbraucherschutz und Lebensmittelsicherheit*) under reference number 33.19.42502-04-16/2272.

References

- Arthur S and Sundaram U (2014) Protein kinase C-mediated phosphorylation of RKIP regulates inhibition of Na-alanine cotransport by leukotriene D(4) in intestinal epithelial cells. *American Journal of Physiology - Cell Physiology* **307**, C1010–C1016.
- Boes J, Kanora A, Havn KT, Christiansen S, Vestergaard-Nielsen K, Jacobs J and Alban L (2010) Effect of *Ascaris suum* infection on performance of fattening pigs. *Veterinary Parasitology* **172**, 269–276.
- CDC (2020) *Parasites – soil-transmitted helminths* [online]. Centers for Disease Control and Prevention. Available at <https://www.cdc.gov/parasites/sth/index.html> (Accessed 10 October 2021).
- Clarke LL (2009) A guide to Ussing chamber studies of mouse intestine. *American Journal of Physiology - Gastrointestinal and Liver Physiology* **296**, G1151–G1166.
- Daines MO and Hershey GK (2002) A novel mechanism by which interferon-gamma can regulate interleukin (IL)-13 responses. Evidence for intracellular stores of IL-13 receptor alpha-2 and their rapid mobilization by interferon-gamma. *Journal of Biological Chemistry* **277**, 10387–10393.
- Dawson HD, Beshah E, Nishi S, Solano-Aguilar G, Morimoto M, Zhao A, Madden KB, Ledbetter TK, Dubey JP, Shea-Donohue T, Lunney JK and Urban JF Jr. (2005) Localized multigene expression patterns support an evolving Th1/Th2-like paradigm in response to infections with *Toxoplasma gondii* and *Ascaris suum*. *Infection and Immunity* **73**, 1116–1128.
- Dold C and Holland CV (2011) *Ascaris* and ascariasis. *Microbes and Infection* **13**, 632–637.
- Donowitz M and Li X (2007) Regulatory binding partners and complexes of NHE3. *Physiological Reviews* **87**, 825–872. doi: 10.1152/physrev.00030.2006
- Easton A, Gao S, Lawton SP, Bennuru S, Khan A, Dahlstrom E, Oliveira RG, Kepha S, Porcella SF, Webster J, Anderson R, Grigg ME, Davis RE, Wang J and Nutman TB (2020) Molecular evidence of hybridization between pig and human *Ascaris* indicates an interbred species complex infecting humans. *eLife* **9**, e61562.
- Guo LY, Hu-Li J and Paul WE (2004) Probabilistic regulation of IL-4 production in Th2 cells: accessibility at the *Il4* locus. *Immunity* **20**, 193–203.
- Harari Y, Russell DA and Castro GA (1987) Anaphylaxis-mediated epithelial Cl⁻ secretion and parasite rejection in rat intestine. *Journal of Immunology* **138**, 1250–1255.
- Henze LJ, Koehl NJ, Bennett-Lenane H, Holm R, Grimm M, Schneider F, Weitschies W, Koziolk M and Griffin BT (2021) Characterization of gastrointestinal transit and luminal conditions in pigs using a telemetric motility capsule. *European Journal of Pharmaceutical Sciences* **156**, 105627.
- Hepworth MR, Danilowicz-Luebert E, Rausch S, Metz M, Klotz C, Maurer M and Hartmann S (2012) Mast cells orchestrate type 2 immunity to helminths through regulation of tissue-derived cytokines. *Proceedings of the National Academy of Sciences of the United States of America* **109**, 6644–6649.
- Hlaing T (1993) Ascariasis and childhood malnutrition. *Parasitology* **107** (Suppl), S125–S136.
- Jungersen G, Eriksen L, Nielsen CG, Roepstorff A and Nansen P (1996) Experimental transfer of *Ascaris suum* from donor pigs to helminth naive pigs. *The Journal of Parasitology* **82**, 752–756.
- Kennedy DJ, Leibach FH, Ganapathy V and Thwaites DT (2002) Optimal absorptive transport of the dipeptide glycylsarcosine is dependent on functional Na⁺/H⁺ exchange activity. *Pflugers Archiv-European Journal of Physiology* **445**, 139–146.
- Klinger S and Breves G (2018) Resveratrol inhibits porcine intestinal glucose and alanine transport: potential roles of Na(+)/K(+)-ATPase activity, protein kinase A, amp-activated protein kinase and the association of selected nutrient transport proteins with detergent resistant membranes. *Nutrients* **10**, 302.
- Klinger S, Lange P, Brandt E, Hustedt K, Schroder B, Breves G and Herrmann J (2018) Degree of SGLT1 phosphorylation is associated with but does not determine segment-specific glucose transport features in the porcine small intestines. *Physiological Reports* **6**, e1356.
- Kocinsky HS, Dynia DW, Wang T and Aronson PS (2007) NHE3 phosphorylation at serines 552 and 605 does not directly affect NHE3 activity. *American Journal of Physiology-Renal Physiology* **293**, F212–F218. doi: 10.1152/ajprenal.00042.2007
- Koehler S, Springer A, Issel N, Klinger S, Strube C and Breves G (2021a) Changes in porcine nutrient transport physiology in response to *Ascaris suum* infection. *Parasites & Vectors* **14**, 533.
- Koehler S, Springer A, Issel N, Klinger S, Wendt M, Breves G and Strube C (2021b) *Ascaris suum* nutrient uptake and metabolic release, and modulation of host intestinal nutrient transport by excretory-secretory and cuticle antigens *in vitro*. *Pathogens (Basel, Switzerland)* **10**, 1419.
- Leles D, Gardner SL, Reinhard K, Iniguez A and Araujo A (2012) Are *Ascaris lumbricoides* and *Ascaris suum* a single species? *Parasites & Vectors* **5**, 42.
- Madden KB, Whitman L, Sullivan C, Gause WC, Urban JF Jr., Katona IM, Finkelman FD and Shea-Donohue T (2002) Role of STAT6 and mast cells in IL-4- and IL-13-induced alterations in murine intestinal epithelial cell function. *Journal of Immunology* **169**, 4417–4422.
- Madden KB, Yeung KA, Zhao A, Gause WC, Finkelman FD, Katona IM, Urban JF Jr. and Shea-Donohue T (2004) Enteric nematodes induce stereotypic STAT6-dependent alterations in intestinal epithelial cell function. *Journal of Immunology* **172**, 5616–5621.
- Masure D, Wang T, Vlaminck J, Claerhoudt S, Chiers K, Van den Broeck W, Saunders J, Vercruyse J and Geldhof P (2013) The intestinal expulsion of the roundworm *Ascaris suum* is associated with eosinophils, intra-epithelial T cells and decreased intestinal transit time. *PLoS Neglected Tropical Diseases* **7**, e2588.
- Mejer H and Roepstorff A (2006) *Ascaris suum* infections in pigs born and raised on contaminated paddocks. *Parasitology* **133**, 305–312.

- Nejsum P, Roepstorff A, Anderson TJ, Jorgensen C, Fredholm M and Thamsborg SM (2009) The dynamics of genetically marked *Ascaris suum* infections in pigs. *Parasitology* **136**, 193–201.
- Notari L, Riera DC, Sun R, Bohl JA, McLean LP, Madden KB, van Rooijen N, Vanuytsel T, Urban JF Jr., Zhao A and Shea-Donohue T (2014) Role of macrophages in the altered epithelial function during a type 2 immune response induced by enteric nematode infection. *PLoS One* **9**, e84763.
- O’Lorcain P and Holland CV (2000) The public health importance of *Ascaris lumbricoides*. *Parasitology* **121**(Suppl), S51–S71.
- Park SJ, Kwon SG, Hwang JH, Park DH, Kim TW and Kim CW (2015) Selection of appropriate reference genes for RT-qPCR analysis in Berkshire, Duroc, Landrace, and Yorkshire pigs. *Gene* **558**, 152–158.
- Paul WE and Zhu JF (2010) How are T(H)2-type immune responses initiated and amplified? *Nature Reviews Immunology* **10**, 225–235.
- Roepstorff A, Eriksen L, Slotved HC and Nansen P (1997) Experimental *Ascaris suum* infection in the pig: worm population kinetics following single inoculations with three doses of infective eggs. *Parasitology* **115**, 443–452.
- Schwarz A, Gauly M, Abel H, Das G, Humburg J, Rohn K, Breves G and Rautenschlein S (2011) Immunopathogenesis of *Ascaridia galli* infection in layer chicken. *Developmental & Comparative Immunology* **35**, 774–784.
- Shea-Donohue T, Sullivan C, Finkelman FD, Madden KB, Morris SC, Goldhill J, Pineiro-Carrero V and Urban JF Jr. (2001) The role of IL-4 in *Heligmosomoides polygyrus*-induced alterations in murine intestinal epithelial cell function. *Journal of Immunology* **167**, 2234–2239.
- Sinniah B (1982) Daily egg production of *Ascaris lumbricoides*: the distribution of eggs in the faeces and the variability of egg counts. *Parasitology* **84**, 167–175.
- Solano-Aguilar G, Shea-Donohue T, Madden KB, Quinones A, Beshah E, Lakshman S, Xie Y, Dawson H and Urban JF (2018) *Bifidobacterium animalis* subspecies *lactis* modulates the local immune response and glucose uptake in the small intestine of juvenile pigs infected with the parasitic nematode *Ascaris suum*. *Gut Microbes* **9**, 422–436.
- Subramanian S, Glitz P, Kipp H, Kinne RK and Castaneda F (2009) Protein kinase-A affects sorting and conformation of the sodium-dependent glucose co-transporter SGLT1. *Journal of Cellular Biochemistry* **106**, 444–452.
- Thamsborg SM, Nejsum P and Mejer H (2013) Impact of *Ascaris suum* in livestock. In Holland C (ed.), *Ascaris: The Neglected Parasite*. Amsterdam: Elsevier, pp. 363–381.
- Thodeti CK and Sjolander A (2002) Leukotriene D4-induced calcium signaling in human intestinal epithelial cells. *Advances in Experimental Medicine and Biology* **507**, 187–191.
- Thwaites DT, Ford D, Glanville M and Simmons NL (1999) H⁺/solute-induced intracellular acidification leads to selective activation of apical Na⁺/H⁺ exchange in human intestinal epithelial cells. *Journal of Clinical Investigation* **104**, 629–635.
- Watanabe C, Kato Y, Ito S, Kubo Y, Sai Y and Tsuji A (2005) Na⁺/H⁺ exchanger 3 affects transport property of H⁺/oligopeptide transporter 1. *Drug Metabolism and Pharmacokinetics* **20**, 443–451. doi: 10.2133/dmpk.20.443
- Zhao H, Wiederkehr MR, Fan L, Collazo RL, Crowder LA and Moe OW (1999) Acute inhibition of Na/H exchanger NHE-3 by cAMP. Role of protein kinase a and NHE-3 phosphoserines 552 and 605. *Journal of Biological Chemistry* **274**, 3978–3987. doi: 10.1074/jbc.274.7.3978
- Zhao A, McDermott J, Urban JF Jr., Gause W, Madden KB, Yeung KA, Morris SC, Finkelman FD and Shea-Donohue T (2003) Dependence of IL-4, IL-13, and nematode-induced alterations in murine small intestinal smooth muscle contractility on STAT6 and enteric nerves. *Journal of Immunology* **171**, 948–954.
- Zhou C, Li M, Yuan K, Deng S and Peng W (2012) Pig *Ascaris*: an important source of human ascariasis in China. *Infection, Genetics and Evolution* **12**, 1172–1177.