Final report

Project title

Understanding the aetiology of acquired equine polyneuropathy by studying Schwann cell cultures

Project number: H-18-47-409

Project period: 2019-2023

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Part 1: Detailed summary

Prosjektets hovedmål var å lage en robust platform for å kunne studere de histopatologiske forandringene i Schwannske celler (SC) hos hester affiserte med acquired equine polyneuropathy (AEP). Cellekulturer fra friske og syke hester skulle settes opp og sammenlignes under forskjellige forhold.

En metode for kultivering av SC fra hest ble etablert. Nerveprøver fra 10 friske og 3 syke hester ble tatt ut. Flow cytometri ble brukt for å skille mellom SC og fibroblaster. Celler fra friske hester ble inkubert med serum fra friske og syke hester for å studere mulig stressrespons. Serum fra 6 hester som var syke med AEP i prosjektperioden og fra en serumbank med friske og syke hester ble analysert med ELISA for IgG och IgM antistoff mot spesifikke gangliosider. Immun-fluorescens og immun-elektronmikroskopi av frysesnitt fra nerver ble undersøkt for påvisning av gangliosid GM1 og GM2.

Prosjektet ble forsinket på grunn av pandemi og flytting av Veterinærhøgskolen. I tillegg ble gjennomføringen vanskeliggjort av at det var færre registrerte tilfeller av AEP i perioden og først i slutten av studieperioden fikk vi tilgang til nerveprøver fra en akutt syk hest med påviste inklusjoner i SC.

Kultivering av SC fra friske og syke hester var vellykket og vi har nå slike cellekulturer nedfrosset. Preliminære resultater fra studier med inkubering av cellekulturer med serum fra friske og syke hester tyder på mer celledød med sykt serum. Hester fra staller med AEP har høyere nivå i serum av IgM antistoff mot spesifikke gangliosider enn friske hester. Vi påviste gangliosid GM1 og GM2 på hestenerver. Vi har nå etablert metodene og har en bank med materiale til å gjenta forsøkene med flere kombinasjoner av friske og syke nerveceller og serum. Videre studier av antistoff mot gangliosider i nerver fra syke hester vil kunne gi informasjon om en potensiell autoimmun rolle i etiologien til AEP. Det vil bli gjort videre studier av cellekulturen fra den syke hesten med inklusjoner i SC.

Metode for uttak av nerveprøve i felt muliggjør cellekulturer fra flere AEP hester. Forhøyede nivåer av IgM antistoffer mot gangliosider må undersøkes videre, men har potensiale for å bli en biomarkør for AEP på stallnivå.

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Pert 2: Main report (max. 10 pages)

Introduction

Acquired equine polyneuropathy (AEP) was first observed in Norway in 1995. Since then, more than 600 cases have been identified throughout Norway, Sweden and Finland. In 2019 there was an outbreak with highly suspicious cases also in Iceland.
Our research over the last 25 years has resulted in several publications (1, 2, 3, 4, 5) as well as a PhD thesis (6), mostly financed through the Swedish-Norwegian Foundation for Equine Research with partners. We have disclosed not only a highly characteristic clinical picture in affected horses, but also homogenously characteristic histopathological changes in peripheral nerves, not described in any other known disease in man or animals (3).
The unique histopathological abnormalities include an inflammatory de- and remyelinating large fibre polyneuropathy, paralleled with hypertrophy of the perikarya (the cytoplasm around the nucleus) as well as conspicuous perinuclear inclusion bodies in the myelin sheath-forming Schwann cells (SC) (3, 7). We showed that the inclusions were not membrane bound, but they stain immunopositive for BiP/GRP78, suggesting that they may be a result of defective posttranslational protein processing in the rough endoplasmic reticulum (rER) (8). BiP/GRP78 belongs to the peptide-binding molecular chaperones that bind newly synthesized proteins and maintain them in a state competent for subsequent folding. Its synthesis is markedly induced under conditions that lead to aggregation of unfolded proteins. The initiating trigger(s) of AEP may therefore directly interfere with the protein folding, or the degradation mechanism, or incite cellular conditions that induce misfolding. Although there is a strong indication that the inclusions consist of protein accumulation, detailed information is still lacking due to previous difficulties in isolating these minor-sized inclusions from biopsy material.
The histopathological findings suggest a primary disruption of Schwann cell metabolism, leading to an inclusion body Schwannopathy, resulting in a secondary attack by the immune system against the myelin sheath. Autoimmune responses to misfolded proteins have previously been reported both in experimental settings and natural diseases, such as sporadic inclusion body myositis in humans (1, 8, 9, 10). The role of the inflammatory changes seen in AEP cases is presently unclear, but autoimmunity may play a role. In general, autoimmune/immune mediated peripheral neuropathies develop when immunologic tolerance to key antigenic sites at components of the peripheral nerves is lost (11). The main interest of research are gangliosides, which are sialic acid-containing glycosphingolipids, because they are abundant and exposed in the peripheral nerves. We have modified a human enzyme-linked immunosorbent assay (ELISA) method and analyzed anti-ganglioside antibodies in serum from horses. We found that serum from AEP-affected horses were positive for IgM-antibodies against a specific ganglioside (unpublished data, Final report SHF H-14-47-014, NFR 248341/E50). These results indicated that serum from affected horses possibly could affect nerves directly, a situation that spurred us to further studies.

Acquired equine polyneuropathy is a devastating neurologic disease in horses and despite years of research, a known aethiology is still lacking. To close in on the aetiopathogenesis, further studies of the development of the histopathological changes in the nerves are necessary. The exact cause and the consequences of the described Schwannopathy unique to AEP has still to be elucidated and may be the key to the aethiology of the disease. A major

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challenge, which has restricted the possibility of further investigations, is the limited availability of AEP nerve samples as these need to be collected in immediate association with euthanasia to avoid postmortem artefacts. Most euthanized case horses are lost to sampling because they cannot be transported alive for euthanasia at a facility with a pathology section, due to the severity of their clinical signs. To overcome the challenge of paucity of study material, without the use of experimental animals, we intended to establish SC cultures of nerves from affected horses and healthy controls. This allows for repeated examinations of the cells, including exposure to putative triggering factors.

Our main aim for this study were to establish a platform for studies of pathological changes in equine Schwann cells. We also intended to follow up our earlier findings of increased levels of anti-ganglioside immunoglobulins in horses during outbreaks of AEP as well as investigate the presence and localisation of their counterpart gangliosides in healthy equine nerves. We intended to set up cell lines with pathological SC from horses affected with AEP, and SC from healthy (non-neurologic) controls from other farms.

The study plan included establishing of a method for culturing equine SC, including the harvesting of nerve material in a field situation, then setting up cell lines in a biobank with cell cultures from both non-neurologic and AEP affected horses. Such cell lines could thereby be studied with different methods and in different environments, such as after exposure to auto-antibody in serum from AEP-affected horses with known levels of anti-ganglioside antibodies, possibly identifying the triggering factor(s) of the pathological changes.

Material and methods

Samples

Serum samples from 6 cases of AEP were collected and added to the previous serum collection during the study period, which has resulted in a biobank of 130 samples from farms affected with AEP, that was used for anti-ganglioside analyses. The horses at AEP farms were symptomatic or asymptomatic at the historical sampling for the biobank. Nerve samples for SC cultivation and other analyses was collected from 4 cases of AEP (resulting in 3 successful SC harvests) and 14 non-neurologic horses, of which cultivation and collection of SC for the biobank has been successful in 10.

SC culturing

In the attempt to establish primary Schwann cell cultures from adult horses, peripheral nerves were harvested from horses at various time points after euthanasia. Nerve tissue from the left deep fibular nerve was obtained under aseptic conditions shortly after euthanasia and placed in ice-cold Leibovitz`s L-15 medium containing gentamicin. The same nerve on the opposite pelvic limb was collected in situ with its muscle tissue, wrapped in surgical drape and placed at 4^0 C for 14,5-19 hours before it was placed in L-15 medium containing gentamicin. In a cell laboratory, Schwann cells were isolated from both left and right deep fibular nerve by using already published protocols for extracting the same cell type from other species. The first step involved mechanical dissociation of the nerve by using forceps under a stereo microscope. Muscle tissue and epineurium were removed, and the nerve fascicles were separated into individual fibers. Subsequently, the nerve fibers were placed in an enzyme solution to promote cell release. After several hours of enzymatic treatment, the tissue was

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fully digested, and the solution containing the released cells was plated onto cell culture vessels. Fresh growth medium was added to the culture 1-3 days after plating. When the cell cultures reached 70-80 % confluency, the cells were passaged or frozen. The cells were detached from the vessel by adding trypsin and collected by centrifugation. For subcultivation, cells were resuspended in growth medium at an optimal density before replating onto new vessels. For cryopreservation, cells were resuspended in freezing medium at a density of 1.0-1.5 x 10^6 cells/ml and stored in liquid nitrogen.
Cells from 4 non-neurological horses were analyzed by flow cytometry to assess the Schwann cell purity in cultures from nerves harvested at different time points after euthanasia. The adherent cells were collected by trypsinization and centrifugation and washed in DPBS. Zombie Violet Fixable Viability Kit was used to exclude dead cells. Further, the cells were incubated with primary antibodies against CD90 (1:20, A15794, ThermoFisher) (fibroblast marker) and CD271 (1:15, AFC-6XPNF0, NordicSite) (Schwann cell marker). Both primary antibodies were fluorophore-conjugated (CD90-PE and CD271-APC). Cytoflex XL (Beckman Coulter) and Kaluza Software (Beckman Coulter) were used to analyze the cells.

Cultivation of cells from non-neurological horses in serum from AEP affected horses
Non-neurological horse cells were divided into three groups and cultured in growth medium supplemented with 10 % serum from a non-neurological horse, an AEP affected horse or FBS. The cell cultures were maintained at 37ºC in a 5 % CO_2 incubator for 2 days.
To investigate the distribution and expression of BiP/GRP78, cells were cultivated in 4 well chamber slides for fluorescence microscopy. The cells were fixed in 4 % paraformaldehyde for 15 minutes, washed with PBS and blocked for 30 minutes in 2 % Bovine Serum Albumin in PBS containing 0.2 % Tween, 7 % glycerol and 2 % goat serum. Cells were incubated with rabbit anti-GRP78 BiP (1:500, ab21685, Abcam) primary antibody for 1 hour in room temperature. Thereafter, the cells were washed with PBS before incubation with anti-rabbit IgG secondary antibody conjugated to Alexa Fluor 594 (1:400, A110006, Invitrogen). The chamber slides were washed with PBS and mounted with ProLong Gold mountant with DAPI (Invitrogen, P36935). The fluorescence signal was examined by using an Axio Imager 2 microscope.
The detection of apoptosis in cell cultures exposed to serum from AEP affected horses was measured by using an Annexin V-staining Kit (ab228554, Abcam) and flow cytometry. The cells were collected by trypsinization and centrifugation, washed with PDBS, and mixed with binding buffer, Annexin V-FITC and Propidium iodide (PI). The cells were incubated for 5 minutes at room temperature in the dark. Cytoflex XL (Beckman Coulter) and Kaluza Software (Beckman Coulter) were used to analyze the fluorescence signal.

Immunolocalization of gangliosides in equine peripheral nerves
To establish the presence and localization of gangliosides in equine peripheral nerves, immunolocalization on frozen sections were used. Samples from the sciatic and deep fibular nerve from non-neurological horses were collected immediately after euthanasia. Samples for fluorescence microscopy were frozen in liquid nitrogen. Samples for immune-electron microscopy were fixed in 4 % paraformaldehyde and 0.1 % glutaraldehyde in Sorensen's phosphate buffer (0.1 M, pH 7.4) over-night.
For fluorescence microscopy, 7 µm cryosections from the sciatic nerve (n=3) and deep fibular nerve (n=2) were cut on a cryostat, placed on glass slides and stored at –80 °C until staining. Samples were fixed in formal calcium (pH 7.3) for 12 minutes, washed in phosphate buffered saline (PBS) and blocked with 2 % goat serum in PBS for 30 minutes. Slides were incubated

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with primary antibodies diluted in PBS for 1 hour and washed with PBS. Thereafter, slides were incubated with secondary antibodies, washed with PBS and mounted with Prolong Gold mountant with DAPI (Invitrogen, P36935). Antibodies used were anti-GM1 (1:50, Bioss, BS-2367R), anti-GM2 (1:50, Novus biologicals, NBP2-81278), anti-neurofilament (1:500, Sigma-Aldrich, N4142), Alexa 488 anti-mouse IgG (1:400. Molecular Probes, A-11029) and Alexa 594 anti-rabbit (1:400, A110006, Invitrogen). Images were taken using an Axio Imager 2 microscope equipped with an Axiocam 506 mono camera (Zeiss).
For immuno-electron microscopy, samples from the deep fibular nerve (n=2) and sciatic nerve (n=1) were embedded in 12 % gelatine, stained with 1 % toluidine blue, cut into small pieces and incubated with 2.3 M sucrose for 20 hours on a rotating wheel at 4 °C. After mounting on cryopins and freezing in liquid nitrogen, 70 nm section were cut on a cryo-ultramicrotome (Leica EM UC6 equipped with FC7 cryochamber). A loop with 1.15 M sucrose and 1 % methylcellulose was used to pick up the sections and place them on formvar- and carbon-coated copper grids (200 mesh, Electron microscopy sciences). Immunolabelling was performed as previously described (12). Primary antibody used was anti-GM1 (1:10-1:400, Bioss).

ELISA for anti-ganglioside IgG and IgM in serum
IgM and IgG antibodies to 6 gangliosides (MAG, GM1, GM2, GD1a, GD1b, GQ1b) were analyzed with a modified ELISA (Bühlmann GanglioCombi MAG ELISA, Bühlmann Laboratories AG, Switzerland) in 54-118 sera from healthy control horses at 118 farms without AEP, and 49-279 sera from horses at 30 farms affected with AEP. Kit calibrators (positive control) and negative controls were prepared with provided incubation buffers as per the kit instructions. Equine serum samples including a high and a low internal control were diluted 1:200 in the kit incubation buffer. For equine samples, the IgM-conjugate was goat anti-horse IgM (Invitrogen TermoFisher Scientific) in 1:20 000 dilution, and the IgG-conjugate was goat anti-horse IgG (Jackson Immunoreseach) in 1:10 000 dilution. 100 µl of equine serum or controls was added to each well and incubated for 120 +/-5 min at 2-8°C, then washed in cold washing buffer. 100 µl of conjugate was added to each well and incubated for 120 +/-5 min at 2-8°C, and subsequently washed with cold washing buffer. 100 µl of TMB at room temperature was added to each well, the plates were sealed, and incubated 30 min at RT while shaken at 400-600 rpm. 100 µl of stop solution was added. The optical density (OD) at 450 nm was analyzed in a spectrophotometer. Cut-off for positive reaction was mean OD + 2SD of controls.

Results and discussion

Case availability
There have been markedly less registered cases of AEP in later years than during the decades before. In 2019 there were 6 cases in Norway, 3 of which were euthanized. In Sweden there were 3 suspected cases. In 2020 there were 8 cases in Norway, 5 of which were euthanized. In Sweden there were 3 suspected cases. For the first time since 1995, there were no cases registered in neither 2021 nor 2022 in Norway. In Sweden there were no cases in 2021 and 2 suspected cases in 2022 of which one was confirmed by video. There have been 2 Swedish cases so far in 2023. In Norway there has so far been one case, which was euthanized in the end of March due to acutely worsening of clinical signs, almost 2 months after the first observations of knuckling. From this case we could collect both nerve samples and serum. We also collected serum from 4 of the 5 euthanized horses in 2020, and one case in 2022, but due

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to long travelling distance, the acute course to euthanasia and the pandemic, we were not able to harvest nerve samples from the euthanized cases in 2019 and 2020.

SC culturing

We have successfully established Schwann cell cultures of nerves from adult horses. In the period 2019-2023, samples were obtained and cultured from 18 horses, out of which 13 cultures were successful. Three of these successful cultures originated from AEP affected horses as follows: Three horses which had previously been affected with AEP 1 (2 horses) or 2 (1 horse) times have been euthanized due to other reasons during the project period. Histopathological findings on peripheral nerve samples from all 3 horses were described as subtle and chronic demyelinating neuropathy without SC inclusions, compatible to horses recovered from AEP. The first horse was in January 2019 and was also the first time cultivating SC from horse was attempted. Unfortunately, this attempt failed. Cultivating of SC from the latter 2 horses succeeded, resulting in 49 million cells to our biobank. The acute Norwegian case from 2023 displayed severe histopathological changes compatible with AEP, including SC inclusions. Cultivation of SC has been successful from this horse, with the collection of 19 million cells kept in liquid nitrogen in our biobank.
Establishing Schwann cell cultures from nerves that have been stored at 4^{o}C for up to 19 hours after sampling has also been successful, enabling harvesting of nerve samples in the field in future cases. The cultures consisted of varying proportions of fibroblasts and Schwann cells, and the images below show how the cells appear in a phase contrast microscopy.

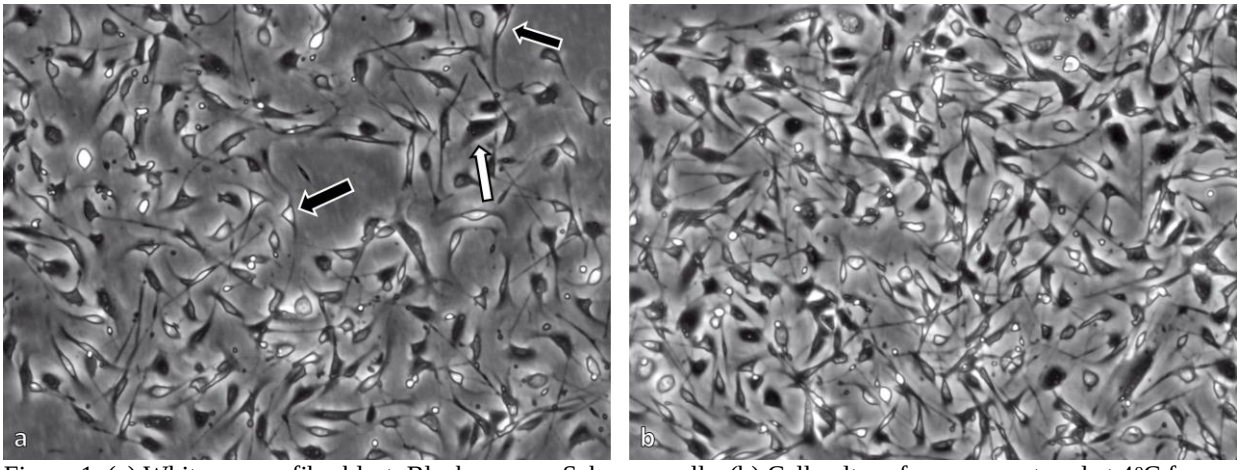

Figure 1. (a) White arrow: fibroblast. Black arrows: Schwann cells. (b) Cell culture from nerve stored at 4^{o}C for 19 hours after sampling.

The cell cultures were analyzed using flow cytometry to confirm the identity of the cells and to assess the purity of the cultures. CD271 was used as a Schwann cell marker and CD90 as a fibroblast marker. As the technical skills of the operators improved, the purity increased and consisted predominantly of Schwann cells.
To separate and isolate Schwann cells from the mixed cultures, we intend to use fluorescence-associated cell sorting (FACS). This is a type of technique that utilizes fluorescent dyes that selectively bind to specific target molecules within the cell or at the cell surface, using an antibody directed against this target molecule. Finding antibodies (anti-CD90 for fibroblasts and CD271 for Schwann cells) which bind specifically to horse cells was challenging. After extensive searching, we have obtained monoclonal antibodies against both CD271 and CD90 that specifically bind to Schwann cells and fibroblasts from horses. This gives us the opportunity to isolate Schwann cells from the cultures if purification is necessary for future experiments.

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Cultivation of cells from non-neurological horses in serum from AEP affected horses
To investigate if serum from horses affected by AEP contains components capable of triggering a stress response in Schwann cells, cells from non-neurological horses were cultured in serum derived from acutely ill horses. For comparison of the distribution and expression of BiP/GRP78 between cultures cultivated in diseased and healthy horse serum, fluorescence microscopy was used. However, no obvious differences were observed. Flow cytometry was used to analyze the extent of apoptosis in the two groups. Preliminary studies suggest a tendency towards a higher proportion of cells in early apoptosis in one out of two affected horses. We intend to carry out additional serum experiments in the future to study apoptosis in cell cultures cultivated in serum from several diseased horses, as well as to investigate the presence of BiP/GRP78-positive inclusions in these cells by using electron microscopy.

Immunolocalization of gangliosides in equine peripheral nerves
The presence of the gangliosides GM1 and GM2 in equine peripheral nerves was established by fluorescence microscopy. GM1 was present along the abaxonal Schwann cell membrane and in the compact myelin with a granular labelling pattern. Furthermore, a strong signal derived from the interface between the adaxonal Schwann cell membrane and axolemma. GM2 was present in the adaxonal Schwann cell membrane and/or axolemma, and in the abaxonal Schwann cell membrane.

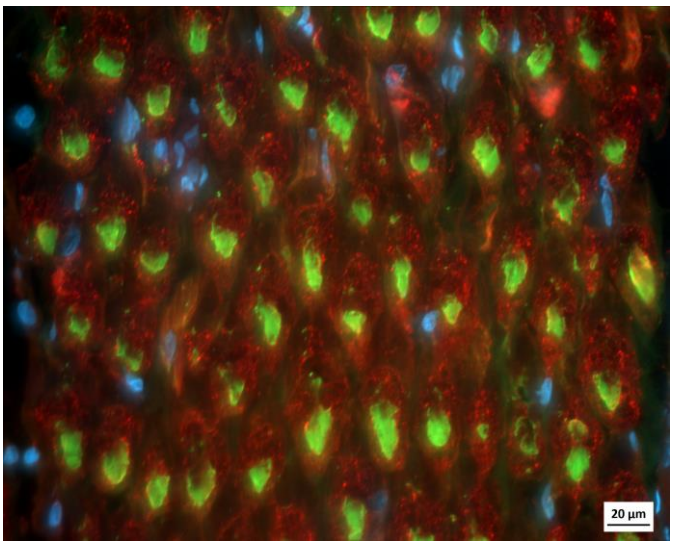

Figure2. Immunofluorescence, equine peripheral nerves, labelled for GM1. GM1 is present in the compact myelin with a granular labelling pattern. Red = GM1. Green = Neurofilament (axonal marker). Blue = Nuclei.

To further investigate the detailed localization of the GM1 in equine nerves, immuno-electron microscopy was used. Signal from the compact myelin was confirmed. Additionally, there was labelling from the apposed axonal and Schwann cell membrane in Remak bundles, and from membranes in organelles within the axons.

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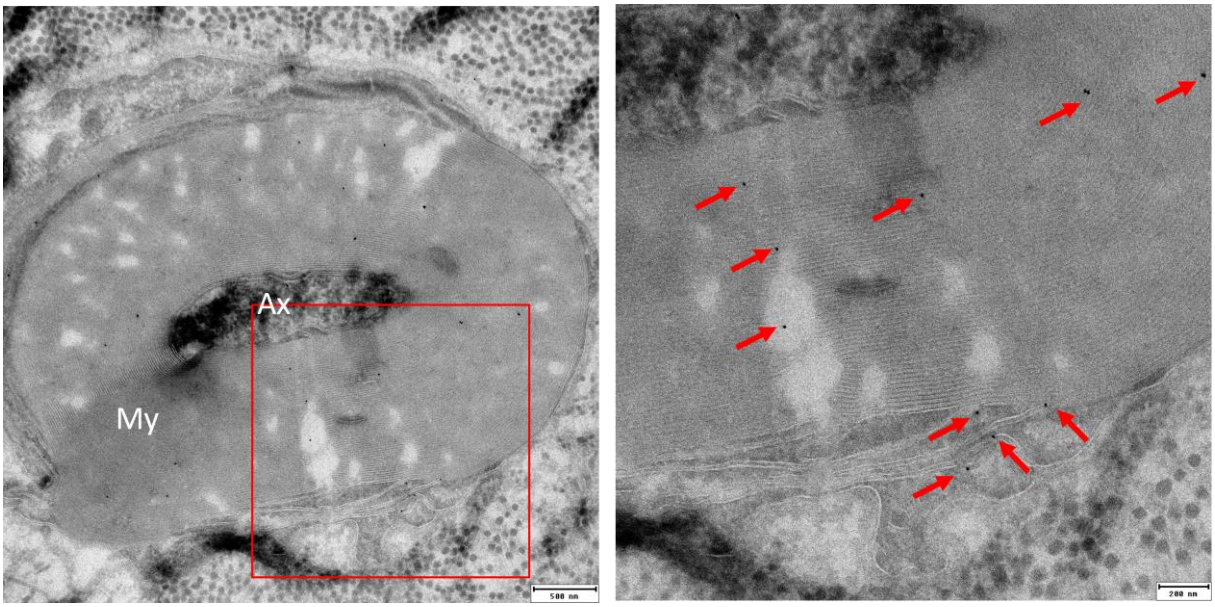

Figure3. Immuno-electron microscopy, equine peripheral nerves, labelled for GM1. Red frame magnified in the image to the right. The black gold particles (10 nm protein A gold, indicated with red arrows) represents the localization of GM1. Signal is present in the compact myelin. My = Compact myelin. Ax = Axon.

To assess the potential role of an immunological reaction against gangliosides in the pathogenesis of AEP, studies investigating the presence of antibodies (IgG and IgM) and complement (C3) in nerves from affected horses are being conducted.

ELISA for anti-ganglioside IgG and IgM in serum

Serum IgM antibodies to all tested gangliosides (MAG, GM1, GM2, GD1a, GD1b, GQ1b) were significantly higher in horses in AEP affected farms compared to control horses ($p<0.05$). 98-100% of these sera were negative for IgG antiganglioside antibodies.

Conclusions

This project has established methods for culturing of equine SC, developed skills for cell sorting of SC and fibroblasts by flow cytometry and demonstrated gangliosides GM1 and GM2 in equine SC membranes. We have hereby provided a stable platform for future research on AEP. A method has even been established for harvesting equine nerves for culturing of SC in field situations, enabling better diagnostics and studies of AEP in the future. A biobank consisting of serum and SC from healthy and AEP affected horses is now available. The presence of gangliosides GM1 and GM2 in equine SC as detected by immunofluorescence and immune-electron microscopy is providing evidence for a target for the significantly increased serum levels of anti-ganglioside IgM antibodies observed in horses in farms with current AEP outbreaks. The results of this study point to possible humoral factors in the aethiopathology for AEP, which will be studied further with the now established new platform.

Relevance for the practical horse sector incl. recommendations

AEP is considered an emerging disease representing serious animal welfare issues, although the prevalence is varying between the years. The successful establishing of equine Schwann cell cultures enable research on the pathological changes in AEP that has not been possible until now. The new knowledge on how to harvest nerve samples for cell cultures in the field makes it possible to collect fresh samples from newly euthanized horses even far from

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established pathology facilities. We have established the presence of gangliosides GM1 and GM2 in equine SC`s membranes, and we have shown increased serum IgM antibodies against certain gangliosides in horses on farms with AEP. Serum anti-gangliosides could possibly be an important biomarker of AEP on farm-level. We have developed methods and collected materials that could be used for further research that will enable us to answer many of the questions around the aethiopathology of AEP.

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Part 3: Result dissemination

Scientific publications, *published*	*Author(s), year, title, journal, Vol, No, pp. (doi/link if applicable)*
Scientific publications, *submitted*	**E. Friis Kvigstad, LK Øverland, F. Strebel Skedsmo, K. Hultin Jäderlund, G. Gröndahl, S. Hanche-Olsen, G. Gunnes. "Cultivation of Schwann cells from fresh and non-fresh adult equine peripheral nerves". J of Neuroscience Methods** https://doi.org/10.1016/j.jneumeth.2023.110054
Scientific publications, *manuscript*	
	Gröndahl, G, et al. "IgM antibodies towards gangliosides are prevalent in Nordic horses with acquired equine polyneuropathy"
Conference publications/ presentations	
	S. Hanche-Olsen. 29th of March 2022. "Acquired Equine Polyneuropathy in Nordic Horses". VetPD Panel Discussion on Mechanical Lameness Case-Discussion **S. Hanche-Olsen. Gardermoen, Norway, 2nd of April 2022. "Acquired Equine Polyneuropathy-an update". Norsk Veterinærkiropraktikerforening.**
	K.H. Jäderlund. Nordic Veterinary Neurology Meeting, Riga, Latvia, May 6th, 2023. **"Antiganglioside antibodies in serum from Nordic horses with acquired equine polyneuropathy".** **K.H. Jäderlund. NMBU, Ås, Norway, May 12th, 2023** **«Antigangliosid-antikroppar i serum från nordiska hästar med acquired equine polyneuropathy».**
	F.S. Skedsmo, K.H. Jäderlund, G. Gunnes, S. Hanche-Olsen, E.F. Kvigstad, K. Matiasek, H. Reineck-Bosaeus, G. Gröndahl **35th ESVN-ECVN symposium, Venice, Italy, Sept 21-23rd, 2023** **"Anti-ganglioside antibodies in Nordic horses with acquired equine polyneuropathy".** **Abstract ID 261 in proceedings, all abstracts will be published in JVIM**
	Acquired Equine Polyneuropathy in Scandinavia. 2019. G. Gröndahl. Dyralaeknafelag. Island

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Other publications, *media etc.*	
Oral communication, *to horse sector, students etc.*	**The neurological horse. G. Gröndahl, November 2020. Webinar for district veterinarians, SVA, Sweden.**
	S. Hanche-Olsen. 4th of March 2022. "Acquired equine polyneuropathy, new research". Nordisk heste- og hundeterapiskole.
	Equine coronavirus & acquired equine polyneuropathy, AEP. G. Gröndahl, May 2022. Seminar for large animal veterinarians, Öland, Sweden.
	The neurological horse. G. Gröndahl, March 2022. Webinar for equine clinicians, Evidensia, Sweden.
Student theses	
	Cultivation of Schwann cells. A tool for investigation of disorders in the peripheral nervous system? I.K. Øverland, F.S. Skedsmo, S. Røed, G. Gunnes. 2020. Forskerlinjeforum
	Devolopment of a cell culture protocol for canine and equine Schwann cells – a tool for the investigation of disorders in the peripheral nervous system. I.K. Øverland. Forskerlinjeoppgave. Veileder G. Gunnes. 2023
Other	

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