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# Acquired equine polyneuropathy of Nordic horses: A conspicuous inclusion body schwannopathy

S. Hanche-Olsen [a,*], K. Matiasek [b,**], J. Molín [b], M. Rosati [b], C. Hahn [c], K. Hultin Jäderlund [a], G. Gröndahl [d]

[a] Department of Companion Animal Clinical Sciences, Norwegian University of Life Sciences, Ullevålsvn 72, 0454 Oslo, Norway
[b] Section of Clinical & Comparative Neuropathology, Centre for Clinical Veterinary Medicine, Ludwig-Maximilians-Universität München, Veterinärstr 13, 80539 Munich, Germany
[c] Neuromuscular Disease Laboratory, Royal (Dick) School of Veterinary Studies, The University of Edinburgh, Easter Bush, Midlothian EH259RG, UK
[d] Department of Animal Health and Microbial Strategies, National Veterinary Institute, 75189 Uppsala, Sweden

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**Abstract**

Acquired equine polyneuropathy (AEP), formerly also known as Scandinavian knuckling syndrome, is one of the most prevalent polyneuropathies in equids in Norway and Sweden, with more than 400 cases registered since first observations in 1995. Despite geographical clustering and an association to forage feeding, its aetiology remains unknown. Clinically AEP is characterized by knuckling due to dysfunction of metatarsophalangeal extensor muscles. This neuropathological study aimed to gain further insights in the pathobiology of AEP and its underlying aetiopathogenesis. We thereby confirmed that all affected horses suffered from similar large fibre neuropathy, exhibiting conspicuous Schwann cell inclusions in most samples, suggestive of a primary disruption of Schwann cell metabolism leading to inclusion body schwannopathy with secondary inflammatory changes. The degree of nerve pathology was not predictive of clinical outcome.


*Keywords:* Knuckling; Schwannopathy; Demyelination; Inclusion body; Inflammatory; Nerve fibre teasing

## 1. Introduction

The first case clusters of a unique neuromuscular syndrome in horses characterized by knuckling in the metatarsophalangeal joints were observed in Norway in 1995 [1] and Sweden in 1998 [2]. Since then, more than 400 cases have been identified throughout Norway, Sweden and Finland, making this disease the most prevalent polyneuropathy in equids in this part of the world [3–7]. The syndrome was associated with peripheral nerve lesions, but the cause has not yet been identified. The disease was initially referred to as "Scandinavian knuckling syndrome", but is now known as acquired equine polyneuropathy (AEP).

AEP affected horses and ponies are of a wide spectrum of breeds, uses and comprise all sexes and ages, except for foals. Clinically, the disease is characterized by digital extensor dysfunction, primarily affecting the pelvic limbs resulting in knuckling in the metatarsophalangeal joints [1,3,4] (Fig. 1). In mild cases, knuckling occurs only rarely unless provoked by e.g. tight circling or sudden stop from trot. Apart from these manipulations of movement, digital extensor dysfunction may be exacerbated with sudden distress, which requires careful handling during clinical examination of more severe cases [4]. Horses with AEP do not appear ataxic. The horses are otherwise alert, responsive with normal appetite and clinical variables are within normal limits. There have been no significant abnormalities on laboratory analysis of blood or cerebrospinal fluid when examined [1,2].

The clinical disease course is highly variable. In the most severe and acute cases, horses suddenly knuckle and stand on the dorsal metatarsophalangeal region without being able to correct the abnormal limb position for seconds to minute(s). Such cases are often unable to get up from recumbency, even with assistance. In less severe and more prolonged disease courses, horses knuckle intermittently for months before they either improve slowly, or suddenly deteriorate and become recumbent. Horses that remain able to rise and stand with or without support mostly recover completely with long convalescence. Intermittent knuckling has, however, been

* Corresponding author.
*E-mail address:* siv.hanche-olsen@nmbu.no (S. Hanche-Olsen).
** Corresponding author.
*E-mail address:* kaspar.matiasek@neuropathologie.de (K. Matiasek).

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Fig. 1. Bilateral knuckling of the metatarsophalangeal joints in an acquired equine polyneuropathy horse.

observed for up to 17 months after onset, with a median duration of clinical signs of 4.4 months [4]. Case fatality rates vary between outbreaks and range from 29% to 53% [1,4].

Typically, AEP affects more than one, but not all horses in a stable and has a seasonal pattern with most cases appearing during winter and springtime, indicating an environmental trigger [1,4,5]. A specific aetiology has not been associated to AEP despite extensive studies, but almost all cases have been fed wrapped forage, indicating an alimentary risk factor of unclear nature [1,4]. However, analysis of the hygienic, botanical, chemical and microbiological composition of wrapped forage has so far failed to identify a disease causing agent (unpublished data) [4].

Despite the relative large number of cases, the sparse availability of fresh material for peripheral nerve studies has hitherto limited the possibilities to clarify the pathobiology of AEP from the tissue perspective. Post-mortem examination of the nervous system of 22 horses diagnosed with AEP in Norway [1] and a number of horses in Sweden (unpublished) indicated a polyneuropathy, but obtained tissues did not allow for further classification. The only in depth investigation reported was from one single horse from Finland and it revealed schwannopathic features and nerve-fibre invasive inflammation [3]. Whether these lesions are characteristic of AEP remains yet unknown, in particular because this horse also was ataxic [3], which is unusual for the majority of AEP cases [1,4]. Hence, it was the aim of this study to clarify peripheral nerve and muscle changes of an extended series of AEP horses presenting with classical clinical signs in order to approach the underlying pathological mechanisms and aetiological triggers.

## 2. Materials and methods

### 2.1. Included horses

Horses were recruited from outbreaks of AEP reported to the Equine Clinic, Norwegian University of Life Sciences (NMBU) or National Veterinary Institute, Sweden, between 2005 and 2014. In accordance with formerly published diagnostic algorithms [1,4], inclusion criteria were a clinical history of repeated bilateral pelvic limb knuckling without signs of involvement of the cranial nerves or other abnormal clinical signs. Exclusion criteria included: (1) primary musculoskeletal disorders affecting the metatarsophalangeal joint, (2) neuromuscular junction disorders, (3) spinal ataxia or (4) primary muscle disease and other causes of non-neurologic pelvic limb weakness. All horse owners consented for the results to be included in this study.

Table 1
Grading of the severity of clinical signs of acquired equine polyneuropathy.

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|---|---|
| Grade I: | Intermittent knuckling of one or both metatarsophalangeal joints when the horse was exercised or stressed, corrected immediately. |
| Grade II: | Knuckling of one or both metatarsophalangeal joints when exercised or stressed and remaining in that abnormal position >3 seconds. |
| Grade III: | Knuckling of both metatarsophalangeal joints when stressed, unable to run, or collapse of the pelvic limbs while attempting to run. |
| Grade IV: | Recumbency. |

Based on neurological examination by authors (S.H.O., G.G., 12 cases), and veterinary records from the neurological evaluation performed by local veterinarians with or without videos (four cases), the clinical severity of each case was graded at least two times; at onset and at time of sampling, some cases also in between. Severity were graded I–IV according to a semiquantitative grading system established earlier [1] (Table 1, Videos S1 and S2). Biopsies and autopsies were performed for diagnostic reasons.

### 2.2. Sampling

In the cases that were euthanized on humane grounds due to deterioration or an uncertain prognosis, samples were taken at autopsy. Fascicular nerve specimens were taken from one or more of the following sites: recurrent laryngeal nerve, median nerve, lateral digital palmar nerve, femoral nerve, sciatic nerve, tibial nerve, common and superficial peroneal nerve and lateral digital plantar nerve (Supplementary material). If possible, nerves were collected from both sides of the body, particularly in the case of the recurrent laryngeal nerves. Specimens from spinal nerve roots were resected after extensive laminectomy.

Biopsies from appendicular muscles including triceps, extensor carpi radialis, quadriceps (vastus lateralis), tibialis cranialis and/or extensor digitalis longus and gluteal muscles were harvested, and specimens were immediately shipped overnight to the laboratories for processing. Cases that were recovering had at least one skeletal muscle biopsy taken.

### 2.3. Histological processing

#### 2.3.1. Nerve processing

All nerve samples underwent the routine biopsy protocol established at the Neuropathology Laboratory, Ludwig-Maximilians University of Munich (LMU), Germany. It includes (1) paraffin embedding for assessment of epineurial, interstitial and vascular abnormalities, (2) semithin sections,