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Case report

Vacuolar myopathy in an adult Warmblood horse

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Abstract

Histopathological interpretation of semimembranosus muscle samples from an adult Warmblood mare with clinical signs suggestive of exertional rhabdomyolysis and intermittent mild elevations in muscle enzyme activities revealed abundant sarcoplasmic vacuoles in all fibre-types containing fine, apparently proteinaceous debris. Vacuolar contents stained lightly with PAS, but did not appear to contain amylopectate, lipid or acid phosphatase and their periphery was unstained with dystrophin immunohistochemistry. Electron microscopy revealed that vacuoles were not membrane bound. No vacuoles were detected in muscle samples evaluated at post mortem following 4 months of rest. To our knowledge, this is the first report of a presumed primary vacuolar myopathy in a horse.
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1. Introduction

Exertional rhabdomyolysis (ER) in horses is a term used to encompass several heritable and presumed acquired conditions. Clinical signs are recognised either during or after exercise and include reluctance to move, muscle stiffness, unusual gait or lameness and apparent pain. In severe cases, signs include pigmenturia, recumbency and occasionally death. Serum biochemistry reveals mild to marked increases in creatine kinase (CK) and aspartate aminotransferase (AST) activities [1].

More widespread use of muscle biopsy during case investigation has enabled veterinary surgeons and scientists better to characterise horses with ER. In particular, recognition of specific pathological features in muscle from affected horses paved the way to the eventual identification of a specific mutation in the skeletal muscle glycogen synthase gene (*GYS1*) in horses of various breeds with type 1 polysaccharide storage myopathy around the world [2–5]. However despite advances, identifying the precise aetiology and pathogenesis of ER in numerous cases remains problematic and many myopathies in horses are best termed idiopathic.

Further characterisation and identification of additional myopathies in horses will likely be accomplished by continued use of muscle pathological investigation in combination with other techniques. In particular, muscle biopsy enables rapid identification of novel phenotypes that might prompt future investigations using other approaches, such as epidemiology, genetic analysis and cell and molecular biology. In this report, we describe the clinical presentation and novel skeletal muscle pathological features in a horse that presented with a history of mild, intermittent ER.

2. Case report

An 8 year old Czech Warmblood mare, imported to the United Kingdom from the Czech Republic in 2002, was examined approximately a year following purchase,

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because of repeated episodes of signs suggestive of ER. The mare first displayed signs of muscle stiffness after exercise in the spring of that year and despite dietary changes (increasing the energy intake derived from fat), had 2 further episodes with similar signs over the following 10 months. In general, the owner reported the horse as being uncomfortable during/after work on multiple occasions. Serum CK and AST activities were moderately raised during a post-exercise episode when the horse was unwilling to move (day 15), and intermittently mildly-moderately raised when veterinary examination had revealed stiffness and apparent back pain during the following few months up until muscle biopsy (Fig. 1).

Fresh and formalin-fixed semimembranosus muscle biopsy samples were submitted to the Comparative Neuromuscular Diseases Laboratory, Royal Veterinary College. The fresh sample was orientated vertically and snap frozen in optimal cooling temperature compound (OCT, Tissue-Tek, Sakura, UK) by submerging it in isopentane pre-cooled in liquid nitrogen. The formalin-fixed sample was processed routinely by paraffin embedding and sectioning. 8 μm fresh frozen cryosections and 5 μm paraffin sections were evaluated with the following stains, using the methods described by Dubowitz et al. [6]: haematoxylin and eosin, modified Gomori Trichrome, Periodic acid Schiff with and without prior digestion with amylase, oil red O, cytochrome oxidase, succinate dehydrogenase and acid phosphatase. Images were captured with an Olympus BX41 microscope and Micropublisher 3.3RTV digital camera (QImaging) and examined with specialist software (QCapture Pro; QImaging). Multiple immunofluorescent labelling of a single cryosection was performed to identify all fibre types according to our previously published method [7]. An F-actin probe (1:40 dilution in PBS for 1 h, Alexa-Fluor 594-phalloidin, Molecular Probes) was applied to cryosections preincubated with 1% bovine serum albumin for 20 min. Sections were co-stained using an antibody directed against dystrophin (Dy8/6C5, Novocastra, Newcastle Upon Tyne, UK, diluted 1:20 and incubated for 1 h) and visualised with secondary antibody, Alexafluor-488 goat anti-mouse IgG (1:500 dilution for 1 h, A1101, Invitrogen, UK). Fluorescent images were captured with a Leica DM4000B and AxioCam Mrm (Zeiss) camera and examined using AxioVision software (Zeiss).

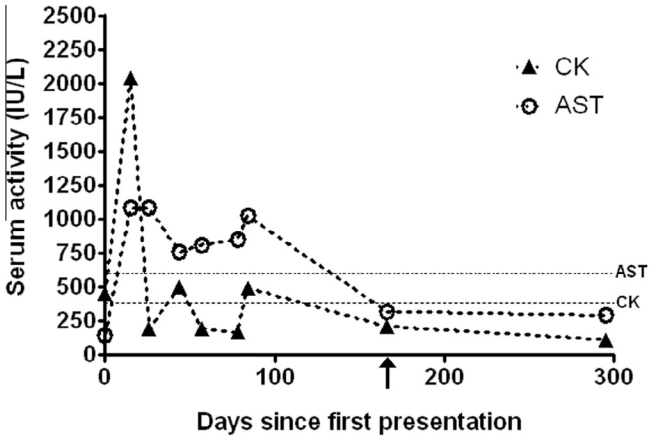

Fig. 1. Serum CK (black triangles) and AST (open circles) activity results over a period of 295 days from first presentation of clinical signs of ER. Maximum normal range for CK activity was 385 iU/L and for AST activity, 600 iU/L as indicated by the horizontal dotted lines. The first muscle biopsy was taken at day 166 (arrow); thereafter the mare was pasture rested prior to euthanasia at day 295.

A portion of the formalin-fixed biopsy was dissected into $<2\ mm^3$ blocks, placed in 4% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.2) for 2 h, rinsed in buffer and stored at 4 °C. Samples were subsequently post-fixed for 1 h in 1% osmium tetroxide in the same buffer, dehydrated through graded ethanols and propylene oxide and embedded in Araldite resin for both transverse and longitudinal sectioning. Ultra-thin sections were prepared, stained with uranyl acetate and lead citrate and imaged using a Megaview III camera (Kodak) on a CM10 transmission electron microscope (Philips).

Histopathology revealed abundant sarcoplasmic small and large vacuoles in both fresh-frozen and formalin-fixed paraffin-embedded (FFPE) sections of semimembranosus muscle (Fig. 2a–c). There was no inflammatory cellular infiltrate or excessive endomyseal or perimyseal fibrosis; occasional internalised nuclei were present. Muscle architecture was significantly compromised in the FFPE samples (Fig. 2c): in these, many vacuoles appeared empty but some vacuoles containing light-purple stained filamentous material on haematoxylin and eosin staining (Fig. 2c). Fibre morphology was better preserved in the fresh frozen sections: a few vacuoles contained small amounts of glycogen, often at the periphery, as indicated by the PAS stain; other vacuoles were devoid of glycogen, or had a similar density of staining seen in the surrounding sarcoplasm (Fig. 2d). There was no amylase-resistant polysaccharide present (Fig. 2e). Vacuolar contents stained light blue with the Gomori trichrome stain (Fig. 2f) and did not stain with mitochondrial histochemical reactions (succinate dehydrogenase; Fig. 2g) or cytochrome oxidase (Fig. 2h). Vacuolar contents were not acid phosphatase positive and no acid phosphatase positive cellular infiltrate was apparent (Fig. 2i). Vacuoles did not contain lipid, neither was there excessive sarcoplasmic lipid seen (Fig. 2j). The vacuole contents were unstained with phalloidin and their periphery was not labelled with an antibody to dystrophin (Fig. 2k). Fibre typing immunofluorescence revealed that vacuoles were present in all fibre types (type 1, 2A and 2X) (Fig. 2l).

Electron microscopy confirmed that the vacuoles were not membrane bound and appeared to displace normal tissue (Fig. 3a and b). As with the paraffin-embedded tissues, the vacuoles were variably filled, however a

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