Cardiac troponin I is a potentially useful test to identify cardiac muscle damage in the horse. Measurements of cardiac troponin I from serum or heparinised plasma samples from 23 clinically normal Thoroughbreds in race training were analysed through a standard Australian commercial laboratory using the ADVIA Centaur® Assay. The cardiac troponin I concentrations were < 0.15 µg/L from all samples. The test was then validated using macerated equine myocardium. Cardiac troponin I concentration may be useful in determining whether poor performance in Thoroughbreds is related to active myocardial disease. 

Until recently the only biochemical tests available for detection of myocardial damage in the horse were isoenzymes of CK (CK-MB) and LDH (LDH1). Both of these have low specificity and sensitivity and are less useful if the animal has concurrent skeletal muscle injury. In human medicine the measurement of serum or plasma concentrations of cTnI, one of the three troponins that form part of the contractile troponin-tropomyosin complex in myocytes, is used for early detection of myocardial damage. In people serum and plasma cTnI levels rise within 12 hours of onset of damage, followed by a plateau phase and a fall to undetectable levels by 5 to 9 days depending on the size of the infarct. In 1997, O’Brien and colleagues established that cTnI was present in equine myocardial tissue at a concentration equivalent to that in human heart muscle and that the equine skeletal muscle activity of cTnI was only 0.05 to 0.1% of the cardiac reactivity level. Cardiac troponin I may therefore also be a potentially useful test in identifying cardiac muscle damage in the horse.

Measurements of equine cTnI are appearing in the literature, however, the methodology has varied in each report. A variety of samples and assays has been used including fresh and frozen serum samples with AXSYM Troponin I® (Abbott Laboratories, Abbott Park, Ill, USA), fresh serum with ADVIA Centaur cTnI assay® (Bayer Corporation, Pittsburgh, PA, USA), frozen serum with Dimension Heterogeneous Immunoassay Module® (Dade-Behring, Newark, DE) and equine myocardial tissue with Stratus I assay® (Dade-Behring, Newark, DE). In routine clinical use in human medicine, there have been marked differences in the clinical performance of various cTnI assays. The findings from one cTnI assay therefore cannot be extrapolated to another unless the assays have been calibrated and have comparable analytical performance at the same clinical decision levels. A difference has also been reported between serum and heparinised plasma in human clinical samples. The purpose of this paper is to report the findings of measurements of cTnI in normal horses from both serum and heparinised plasma analysed through a standard Australian commercial laboratory (Mayne Health Vetnostics).

Material and methods

Blood was collected from two different groups of horses at two different times. The initial group of horses (group A) were randomly selected to assess any differences in cTnI concentrations between sample type. Seven clinically normal Thoroughbreds (five fillies and two geldings) in training were sampled. The horses ranged in age from 3 to 5 years (mean ± SD 4 years ± 0.82). Blood was collected into both lithium heparin and plain blood tubes approximately 3 hours after normal morning track work and sent for immediate testing. A second group of horses (group B) were later randomly collected so we could assess larger numbers of horses. Lithium heparin was the only sample collected as this was the routine sample taken from these horses prior to racing. Sixteen clinically normal Thoroughbreds (eight fillies, seven geldings, one colt) in training were sampled. These horses ranged in age from 2 to 5 years (mean 3.25 years ± 0.89). Blood was collected approximately 3 hours after normal track work. The samples were frozen at −4°C and stored for up to 16 days before analysis, for logistical reasons.

The immunoassay for cTnI was performed using the ADVIA Centaur® Assay (Bayer Corporation, Pittsburgh, PA, USA). The ADVIA Centaur cTnI assay is a two site sandwich immunoassay that uses constant amounts of polyclonal and monoclonal antibodies. The limit of detection of the assay is 0.15 µg/L.

To validate the assay, samples of skeletal muscle, myocardium and serum were collected from a healthy adult Thoroughbred mare that was euthanased because of chronic non-healing of an olecranon fracture sustained some 5 months previously. Ten mL of clotted blood was collected via jugular venipuncture prior to euthanasia and 30 g of each of skeletal muscle and myocardium were collected and stored separately in sterile 70 mL containers within 15 minutes of euthanasia. The samples were chilled to 4°C, the clotted blood was centrifuged at 1500 rpm and the serum was collected within 60 min. All samples were stored at −12°C within 24 hours of collection until processed. One gram of each of skeletal muscle and myocardium were separately...
Results

The concentration of cTnI from all individually measured samples in fresh lithium heparin and fresh serum in group A and frozen lithium heparin in group B were less than 0.15 µg/L; that is, below the limit of detection of the assay.

In the assay validation, total cTnI concentrations of 0.20, 0.30 and 0.35 µg/L were respectively achieved for the undiluted sample, the 1 in 5 dilution and the 1 in 10 dilution of skeletal muscle homogenate. Total cTnI concentrations of > 50.00, 2,541.40 and 2,200.55 µg/L were respectively achieved for the undiluted, 1 in 5 dilution and 1 in 10 dilution samples of the myocardial homogenate. The cTnI concentration in the serum was 0.09 µg/L, below the accurate limit of level of detection of the assay (< 0.15 µg/L).

Discussion

As expected from previous equine studies, the cTnI concentration in these horses, with no known cardiac damage, were below the limit of detection of the assay. These horses were fit Thoroughbreds in active training and were sampled within a week of racing at a metropolitan or provincial meeting. With normal values below the limit of detection of the assay, differences between the type of samples (fresh serum, fresh heparinised plasma, frozen heparinised plasma) were not apparent. In human patients with elevated cTnI concentrations, differences in results can vary, depending on the sample. The ADVIA Centaur® Assay describes a median 11% decrease in sample values in heparinised plasma when compared to serum. When monitoring a patient’s progress it is therefore not recommended that the heparinised plasma or serum samples be used interchangeably. As the cTnI concentrations in the horses from our study were below the level of detection, the comparison between types of samples from Group A and Group B could not be made, and it is possible that differences would become apparent if cTnI concentrations were elevated.

As there is only one other veterinary publication in which this assay was used, validation of the assay was considered necessary. Using macerated myocardium and skeletal muscle and serum it was clearly shown that the ADVIA Centaur cTnI assay was able to detect equine troponin in equine myocardium and that there was minimal cross reaction with skeletal muscle troponin.

A readily available commercial laboratory assay was selected for this study to make the information accessible and thus potentially useful to Australian veterinary practitioners. Only one assay at one laboratory was used to analyse the samples; possible variations between assays and laboratories were not investigated.

In conclusion, we have reported the normal equine cTnI values as < 0.15 µg/L, in fresh serum, fresh heparinised plasma and frozen heparinised plasma as measured in a standard Australian commercial laboratory using a standard assay (the ADVIA Centaur® Assay by Bayer) under routine conditions. It is hoped that identifying elevated serum or plasma concentrations of cTnI may be helpful in the detection of active myocardial damage in the horse, as may occur with recent exposure to myocardial toxins such as monensin. On the basis of the information available from the human literature, the highest measured cTnI concentration would be expected from a fresh serum sample taken 12 hours to 5 days after the onset of myocardial damage.

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