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Research article

# Point-of-care cardiac troponin I in non-domestic species: a feasibility study

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**Abstract**
One of the main challenges faced by veterinary surgeons in the field of zoo and wild animal medicine is the lack of published reference data and validated tests for non-domestic species. In the field of cardiology the diagnostic techniques used for domestic animals are also applied to exotic species. However due to the wide range of species dealt with and their different sizes, few techniques have been validated. Recent advances in human and domestic animal medicine have shown cardiac troponin I (cTnI) to be the biomarker of choice for myocardial damage. The primary aim of this feasibility study was to determine whether the i-STAT®1 hand-held analyser can be used to identify elevations of serum and plasma cTnI in a variety of non-domestic species. The secondary aim was to explore whether elevations in cTnI were related to underlying cardiac pathology. During routine health checks at the Zoological Society of London, 171 blood samples were collected from 36 different species (27 mammal, seven bird and two reptile) and were analysed for cTnI using the i-STAT®1 hand-held analyser. Concentrations of cTnI below, equal to and well above a suggested cut-off of 0.08 µg/L were observed in mammalian species. The majority of animals with concentrations of cTnI above 0.08 µg/L were subsequently shown to have pathology with potential cardiac involvement. All avian and reptilian samples were below 0.00 µg/L even though overt cardiac pathology was noted in some of these animals. In conclusion, the assessment of cTnI using the i-STAT®1 hand-held analyser shows promise as a humoral marker of cardiac pathology in mammalian species but not for avian and reptilian species. Further work is required to define species-specific reference intervals for cTnI.

## Introduction

Underlying cardiac pathology is often responsible for the death of many non-domestic animals (Van Vleet and Ferrans 1986). The use of diagnostic tests such as cardiac ultrasound and electrocardiograms is often precluded due to cost, limited availability of equipment and a lack of recognised reference intervals. Interpretation of results from these tests is complicated further by the diverse anatomy and size of the different species, making interpretation of such tests challenging. Accordingly, reliable point-of-care humoral markers of cardiac health would provide a useful tool in the diagnosis and management of heart disease in non-domestic animals. Cardiac troponin (cTn) is one such marker that has shown promise, being the cornerstone for the assessment of myocardial damage in both humans and domestic species (Spratt et al. 2005; Wells and Sleeper 2008). Cardiac troponin I (cTnI) is specific for the myocardium and is released from damaged cardiomyocytes following any insult that causes cell death (e.g. myocardial infarction, myocarditis, dilated cardiomyopathy, congestive heart failure, cardiotoxic drugs, blunt trauma, cardiac injury in renal failure and sepsis) (Sarko et al. 2002; Collison and Gaze 2005; Patil et al. 2011; Giannitsis and Katus 2013). In healthy animals and humans, circulating cTn is extremely low or undetectable; therefore elevations in circulating cTn convey important diagnostic and prognostic information (Spratt et al. 2005; Nostrell and Häggström 2008; Clerico et al. 2008).

Cardiac troponin I is encoded in the higher vertebrates by orthologous genes (Hastings 1997). Thus it is presumed that cTnI is highly conserved across species. Assays developed for its measurement in human patients have been validated in a number of domesticated species (O'Brien et al. 1997; Rishniw et al. 2004; Adin et al. 2006) and, more recently, for the white-tailed deer (*Odocoileus virginianus*) (Boesch et al. 2015). The epitope detected by the antibodies in the currently available assays is a broad central region of the protein. It has therefore been suggested that the same assay used for humans and domestic species should accurately detect cTnI in non-domestic

**Table 1.** Cardiac troponin I concentrations in birds and reptiles. n = number of samples.

| Common name | Scientific name | n | cTnI µg/L | Relevant clinical and/or post-mortem findings |
|---|---|---|---|---|
| Red billed toucan | *Ramphastos tucanus* | 1 | 0.00 | Healthy at the time of blood sampling |
| Keel billed toucan | *Ramphastos sulfuratus* | 1 | 0.00 | Sudden death post-testing, emaciated |
| Black billed whistling duck | *Dendrocygna arborea* | 2 | 0.00 | Healthy at the time of blood sampling |
| Black swan | *Cygnus atratus* | 1 | 0.00 | Cardiomyopathy associated to lead toxicosis |
| Rockhopper penguin | *Eudyptes moseleyi* | 2 | 0.00 | Cardiac arrhythmias, severe malaria, died shortly after blood sampling |
| Reticulated python | *Python reticulatus* | 1 | 0.00 | Chronic pneumonia |
| Gaboon viper | *Bitis gabonica* | 1 | 0.00 | Sample pre-euthanasia. Pericardial effusion, hypertrophic cardiomyopathy and bleeding of the coronary vessels at post-mortem |

species (Apple et al. 2004). Accordingly, the aims of this feasibility study were two-fold: firstly, to determine whether the i-STAT® 1 point-of-care hand-held analyser can identify circulating cTnI in a large range of non-domestic species, and secondly, if cTnI can be identified, to assess whether there is any relationship between cTnI concentrations and underlying cardiac pathology.

## Methods

Between 2008 and 2010, 171 blood samples were collected opportunistically from 92 individuals from 34 different species (27 mammal, five bird and two reptile) at the Zoological Society of London (ZSL). In total, blood samples were obtained from 83 individual mammals with repeat samples obtained from 30 individuals (2–4 samples). Samples were obtained from seven birds and two snakes with no repeats from any individuals. Of the 27 mammalian species tested, 19 were non-human primates (five prosimians, eight New World monkey, four Old World monkey and two ape species), six were carnivores, one was a rodent and one an elephant. Each sample was analysed using the i-STAT®1 immunoassay following the manufacturer's guidelines (*i-STAT System,* ©2013 Abbott Point of Care Inc.).

The i-STAT® cTnI method is a two-site enzyme-linked immunosorbent assay in which antibodies specific for cTnI are housed on a silicon chip along with an antibody/alkaline phosphatase enzyme conjugate specific to a separate portion of the cTnI molecule. Whole blood or plasma is brought into contact with the sensors, allowing the enzyme conjugate to dissolve into the sample. The cTnI within the sample becomes labelled with alkaline phosphatase and is captured onto the surface of the electrochemical sensor during a seven-minute incubation. The sample and excess enzyme conjugate are washed off the sensor. Within the wash fluid is a substrate for the alkaline phosphatase enzyme. The enzyme bound to the antibody/antigen/antibody sandwich cleaves the substrate, releasing an electrochemically detectable product. The electrochemical sensor measures this enzyme product, which is proportional to the concentration of cTnI in the sample. In a human reference population (n=162 apparently healthy donors) the assay was linear from 0.00 to 50µg/L (https://abbottpointofcare.com, Rev. Date: 01-Jul-13). According to the manufacturer the imprecision was 7.6–8.5% in the range 0.53–31.82 µg/L with a human 99$^{th}$ percentile concentration of 0.08 µg/L to define a positive cTnI. According to a technical brief from the distributor Heska Corporation, the range of cTnI in canine, feline and equine species were 0.00–0.11, 0.00–0.09 and 0.00–0.06 µg/L respectively (http://woodleyequipment.com, 8 September 2015)

Analyses were carried out at the veterinary facilities at ZSL London Zoo and ZSL Whipsnade Zoo. As the test only requires 17 µl of whole blood or plasma, a project licence (within the requirements of the UK Animals (Scientific Procedures) Act 1986) was not required for this investigation as all blood samples used were taken from those generated during routine health-checks or during investigation of clinical disease. Low sample size from each of the different species examined precluded statistical analysis. Based on an average of previous cut-offs identified in human, canine, feline and equine species, individual data from animals presenting with concentrations above 0.08 µg/L are presented. In all of these animals clinical and post-mortem records were checked for potential underlying cardiac pathology.

## Results

Avian and reptilian samples are presented in Table 1; all were undetectable for cTnI (0.00 µg/L). Mammalian data are presented in Table 2, and Table 3 lists those animals presenting with a cTnI concentration greater than 0.08 µg/L (n=22) alongside relevant clinical or post-mortem findings. Of these 22 mammals, 14 had clinical and/or post-mortem findings indicative of cardiac damage. Eight other animals (five Alaotran gentle lemurs *Hapalemur alaotrensis*, one black-tailed silvery marmoset *Callithrix argentata argentata*, one meerkat *Suricata suricatta* and one serval *Leptailurus serval*) with concentrations of circulating cTnI greater than 0.08 µg/L had no clinical signs of cardiac damage at the time of blood sampling.

## Discussion

The purpose of this study was to determine whether the i-STAT®1 point-of-care hand-held analyser could identify circulating cTnI in non-domestic species, and secondly, if circulating cTnI was identified with the i-STAT®1 analyser, whether it was related to underlying pathology. The main findings from this study were: 1) the i-STAT®1 successfully identifies circulating cTnI in a variety of non-domesticated mammals, 2) the i-STAT®1 appears unable to identify cTnI in avian and reptile species, and 3) elevations in cTnI above 0.08 µg/L in mammal samples is probably indicative of pathology with cardiac involvement.

The use of imaging to diagnose cardiac pathology is not always practical in non-domesticated species. Specialised equipment and technical expertise is needed and general anaesthesia is usually required to enable consistent positioning, standardisation of protocols and enhanced image quality. Accordingly, a humoral marker of cardiac pathology that can be measured at the point-of-care is highly desirable. Cardiac troponin measurement is now a cornerstone in the diagnosis of cardiac disease in humans and domesticated animals (Spratt et al. 2005; Wells and Sleeper 2008) and has potential to be a useful tool for veterinary professionals caring for non-domesticated animals. Findings from the present feasibility study suggest that the i-STAT®1 can detect both minor (less than 0.08 µg/L) and significant (more than 0.08 µg/L)

**Table 2.** Cardiac troponin I concentrations in mammals.

| Common name | Scientific name | n | cTnI range (µg/L) | Number of animals below 0.02 µg/L cTnI | Number of animals with 0.02–0.08 µg/L cTnI | Number of animals with >0.08 µg/L cTnI |
|---|---|---|---|---|---|---|
| Asian elephant | *Elephas maximus* | 8 | 0–1.27 | 7 | 0 | 1 |
| Aye-aye | *Daubentonia madagascariensis* | 2 | 0.18–2.22 | 0 | 0 | 2 |
| Alaotran gentle lemur | *Hapalemur alaotrensis* | 7 | 0.02–0.59 | 2 | 0 | 5 |
| Bare faced tamarin | *Sanguinus bicolor* | 1 | 0 | 1 | 0 | 0 |
| Black cap squirrel monkey | *Saimiri boliviensis* | 12 | 0–0.14 | 11 | 0 | 1 |
| Black-tail silvery marmoset | *Callithrix argentata argentata* | 3 | 0.13–0.36 | 0 | 0 | 3 |
| Brazilian agouti | *Dasyprocta leporina* | 1 | 0.01 | 1 | 0 | 0 |
| Chimpanzee | *Pan troglodytes* | 7 | 0–4.21 | 3 | 3 | 1 |
| Diana monkey | *Cercopithecus diana* | 1 | 0.01 | 1 | 0 | 0 |
| Francois langur | *Trachypithecus francois*i | 1 | 0.02 | 1 | 0 | 0 |
| Golden-headed lion tamarin | *Leontopithecus chrysomelas* | 4 | 0.02–0.31 | 2 | 1 | 1 |
| Golden lion tamarin | *Leontopithecus rosalia* | 2 | 0.04–0.22 | 0 | 1 | 1 |
| Goeldi's monkey | *Callimico goeldii* | 4 | 0.05–0.15 | 0 | 3 | 1 |
| Grey-legged douroucouli | *Aotus lemurinus griseimembra* | 1 | 0.04 | 0 | 1 | 0 |
| Hanuman langur | *Semnopithecus* spp. | 2 | 0.04–0.48 | 0 | 1 | 1 |
| Kinkajou | *Potos flavus* | 1 | 0.03 | 0 | 1 | 0 |
| Meerkat | *Suricata suricatta* | 10 | 0–0.33 | 7 | 1 | 2 |
| Potto | *Perodicticus potto* | 2 | 0.01–0.05 | 1 | 1 | 0 |
| Pygmy slow loris | *Nycticebus pygmaeus* | 1 | 0.01 | 1 | 0 | 0 |
| Red-faced spider monkey | *Ateles paniscus* | 1 | 0.02 | 1 | 0 | 0 |
| Ring-tailed lemur | *Lemur catta* | 1 | 0.42 | 0 | 0 | 1 |
| Ring-tailed coati | *Nasua nasua* | 4 | 0.03–0.06 | 0 | 4 | 0 |
| Serval | *Leptailurus serval* | 1 | 0.36 | 0 | 0 | 1 |
| Sulawesi crested macaque | *Macaca nigra* | 1 | 0.01 | 1 | 0 | 0 |
| Sumatran tiger | *Panthera tigris sumatraensis* | 2 | 0–0.15 | 1 | 0 | 1 |
| Western lowland gorilla | *Gorilla gorilla gorilla* | 1 | 0.01 | 1 | 0 | 0 |
| Yellow mongoose | *Cynictis penicillata* | 2 | 0–0.06 | 1 | 1 | 0 |

elevations in cTnI in a wide range of mammalian species. The clinical findings related to the majority of mammals in the present study with a cTnI greater than 0.08 µg/L suggest that this cut-off is potentially suitable across the mammalian species studied. For instance, a golden-headed lion tamarin (*Leontopithecus chrysomelas*) with elevated cardiac troponin I (0.29 µg/L) had a right-sided dilated cardiomyopathy confirmed on radiography and echocardiography. After therapy with ACE inhibitors a marked clinical improvement was noticed, alongside a marked reduction in cTnI (0.07 µg/L). In another case, a juvenile chimpanzee (*Pan troglodytes*) undergoing a health assessment following a bite wound presented with a cTnI concentration of 0.35 µg/L. Two months later the same animal was found collapsed and died shortly after while undergoing treatment. On post-mortem examination it was diagnosed with underlying cardiomyocyte pathology (Tong et al. 2014), which probably corresponded with an ante-mortem cTnI concentration of 4.21 µg/L. The elevation in cTnI in a single elephant (*Elephas maximus*) corresponded with infection and/or transient DNAaemia with elephant endotheliotropic herpes virus (EEHV), which is known to cause endothelial damage and subsequent cardiomyocyte disruption. The two elephant samples were obtained six weeks apart, the first elevated sample concurred with a clinical episode of EEHV and the subsequent lower value corresponded with the animal's recovery and possibly a lack of persistent viral insult to the heart (Richman et al. 2000, Stanton et al. 2013; Ashrafpoor 2013 ).

While the majority of cTnI samples above 0.08 µg/L were linked to potential cardiac pathology, eight animals had unremarkable clinical signs. Of these eight animals, six were juvenile primates (and of these five were Alaotran gentle lemurs *H. alaotrensis*). In humans it has been shown that paediatric cardiac troponin reference intervals are higher than in adult populations (Koerbin et al. 2012; Bailey et al. 2013). Therefore, the same may be true for juveniles of some non-human primate species, although further work is required to confirm this.

Recent advances in laboratory-based cTn assays have shown that there is a low circulation level of cTn in healthy human populations (Wu et al. 2006); it is likely that this is also the case in other mammalian species. Accordingly, it is possible that low-level concentrations of cTnI are not of concern. However, further work is required in large reference populations before species-specific cut-offs can be developed.

**Table 3.** Animals with cardiac troponin I concentrations greater than 0.08 ng/ml alongside clinical findings.

| Common name | Scientific name | n | Individual samples >99th percentile (0.08 µg/L) | Relevant clinical and/or post-mortem findings with cardiac involvement |
|---|---|---|---|---|
| Asian elephant | *Elephas maximus* | 1 | 1.27, 0.25 | Animal positive to elephant endotheliotropic herpes virus |
| Aye-aye | *Daubentonia madagascariensis* | 2 | 0.18<br>2.22 | Grade II heart murmur.<br>Grade II heart murmur. |
| Alaotran gentle lemur | *Hapalemur alaotrensis* | 5 | 0.1, 0.1<br>0.45<br>0.21<br>0.73<br>0.15 | < 1 year old, healthy at the time of testing<br>< 1 year old, healthy at the time of testing<br>< 1 year old, healthy at the time of testing<br>Young adult female, healthy at the time of testing<br>Young adult male, healthy at the time of testing |
| Black-cap squirrel monkey | *Saimiri boliviensis* | 1 | 0.14 | Epileptic very stressed at time of blood sampling. |
| Black-tail silvery marmoset | *Callithrix argentata argentata* | 3 | 0.13<br>0.3<br>0.36 | Juvenile, healthy at the time of blood sampling<br>Sudden death a month post testing<br>Cardiomegaly, emaciated, lymphoma |
| Chimpanzee | *Pan troglodytes* | 1 | 0.35, 4.21 | Sudden cardiac death |
| Golden-headed lion tamarin | *Leontopithecus chrysomelas* | 1 | 0.29, 0.18, 0.19, 0.31 | Congestive heart failure |
| Golden lion tamarin | *Leontopithecus rosalia* | 1 | 0.22 | Gastro-intestinal tympany and renal disease. Cardiac myocyte necrosis at post-mortem. |
| Goeldi's monkey | *Callimico goeldii* | 1 | 0.15 | Death due to leptospirosis with secondary *Pseudomona sp.* septicaemia |
| Hanuman langur | *Semnopithecus* spp. | 1 | 0.48 | Mitral valve disease |
| Meerkat | *Suricata suricatta* | 2 | 0.11<br>0.33 | Healthy at the time of blood sampling<br>Hypercholesterolaemic with liver pathology |
| Ring-tailed lemur | *Lemur catta* | 1 | 0.42 | Cardiomegaly |
| Serval | *Leptailurus serval* | 1 | 0.31 | Healthy at the time of blood sampling |
| Sumatran tiger | *Panthera tigris sumatraensis* | 1 | 0.15 | Severe bite wounds at the time of blood sampling. |

The only stimulus for elevations in cTn in humans that is not related to poor cardiac outcome is strenuous exercise (Shave et al. 2010). This exercise-induced release of cTn has also been observed in racing dogs (Tharwat et al. 2013a), horses (Nostrell and Häggström 2008) and camels (Tharwat et al. 2013b) and could be anticipated in other mammals. If so, then physical activity prior to sample collection may result in slight elevations in cTn that do not indicate cardiac pathology. In this study the positive cTnI concentrations in both the Sumatran tiger (*Panthera tigris sumatraensis*) and black-capped squirrel monkey (*Saimiri boliviensis*) may be explained by stress-related activity (conspecific aggression with subsequent trauma and epileptic seizures respectively) prior to sedation. Additional work is required to explore the relationship between circulating cTn and activity in non-domesticated animals. Until this is done veterinary professionals dealing with non-domesticated species should be aware that intense activity, such as that observed during anaesthetic darting, may result in slight elevations in cTn and should interpret modest elevations within this context. It is important to note that two animals in the present study (serval and meerkat) had elevations in cTnI that cannot, at the time of writing, be explained by cardiac pathology, stress/exercise or age. However we are aware that they may yet present with frank cardiac disease.

In contrast to the mammals investigated, none of the samples obtained from avian or reptile species were positive for cTnI. The interpretation could be that none of these animals had underlying cardiac pathology. However, several individuals were known (and confirmed on post-mortem examination and subsequent histopathology) to have severe myocardial damage and cardiac disease. As an example, an adult Gaboon viper (*Bitis gabonica*) that had a cTnI of 0.00 µg/L prior to euthanasia was shown to have pericardial effusion, hypertrophic cardiomyopathy and bleeding of the coronary vessels on post-mortem examination. Similarly, an adult black swan (*Cygnus atratus*) suffering from severe lead toxicosis and associated cardiomyopathy also had undetectable cTnI levels. Accordingly, the negative cTnI data in the birds and reptiles more likely reflects differing cTnI epitopes between mammals, birds and reptiles. There is only a 60% homology between the chicken and human cTnI epitope sequence (NCBI Blast software; http://www.ncbi.nlm.nih.gov/; http://blast.ncbi.nlm.nih.gov/). As such the i-STAT®1 assay may not be sensitive to elevations in cTnI in either birds or reptiles. Therefore, until avian and reptilian specific antibodies are developed, assessing cTnI with the i-STAT®1 cannot currently be recommended.

The cTn assay for the i-STAT®1 is not validated yet for zoo and most wild animal species, but these preliminary results are promising. cTnI may prove to be a very useful tool for the diagnosis, prognosis and monitoring of cardiac disease in some of the species examined. A recent study carried out by Boesch et al. (2015) in the white-tailed deer (*Odocoileus virginianus*) validated the assay for this species, thereby offering encouragement for its use in other wild animals. In addition it appears that cTnI measurement may be useful in the assessment of cardiac damage secondary to other disease states such as septicaemia or renal compromise. It is important to note that cardiac troponin I analysers have not been standardised and because each vendor

may use different antibodies to varying epitopes in the assay, values obtained on one assay may not be directly comparable to values obtained on a different assay. Therefore, serial comparisons of cTnI in the same animal using different assay platforms are not valid. It is recommended that individual collections standardise their approach to the assessment of cTn and if possible generate their own reference intervals for the species under examination. The results from this feasibility study show promise, but there is a clear need for larger studies in order to set species-specific reference intervals.

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