Assay of cardiac troponins in the diagnosis of myocardial degeneration due to foot-and-mouth disease in a calf

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TROPONIN, a globular protein localising on the thin filament of all striated muscles, plays a role in the contraction and relaxation of striated muscles. Cardiac troponins consist of three subunits: cardiac troponin-T (cTn-T), cardiac troponin-I (cTn-I) and troponin-C (cTn-C) (Cardinet 1997, Müller-Bardorff and others 1997, Rice 1999). The diagnostic and prognostic importance of troponins has been determined in human beings with myocardial infarction (MI). Since they are released during microinjuries to the heart muscle, cTn-I and cTn-T have been determined to be better cardiac markers in MI than other markers such as creatine kinase-MB (CK-MB), aspartate aminotransferase (AST), lactate dehydrogenase (LDH) and myoglobin (Müller-Bardorff and others 1997, Azzazy and Christenson 2002).

cTn-I is an excellent candidate biomarker of cardiac injury in all mammalian species. O'Brien and others (1997) demonstrated that the reactivity of cTn-I is higher for myocardium than for skeletal muscle, and there is a marked sequence homology between human and the bovine cTn-I. cTn-T is another cardiac structural protein released into the circulation during myocardial injury, and although it is a valuable cardiac marker, it is no more sensitive than CK-MB for acute MI in human beings (Jurlander and others 2000, Koukkunen and others 2001); it is also released during skeletal muscle and renal damage (Mockel and others 1999).

Myocarditis in domestic animals may result in sudden death. In young calves, one cause of sudden death is foot-and-mouth disease (FMD), a contagious disease of ruminants with high morbidity. Although the mortality rate is considerably lower in adult cattle, in calves it may be up to 50 per cent: young calves may die before showing any observable lesions due to virus-induced damage to the developing cells of the myocardium (Van Vleet and Ferrans 1986, Barnett and Cox 1999).

This short communication describes a study to assess the value of human cTn-I and cTn-T kits for the diagnosis of myocardial degeneration due to FMD in calves.

A three-month-old Brown Swiss bull calf weighing 60 kg was admitted to the clinic of the Faculty of Veterinary Medicine, University of Kafkas, Turkey, on March 13, 2003. The calf was comatose. The case history revealed that cattle on the same farm were suffering from FMD and another calf had been found dead the previous day. Physical examination of the presented calf was carried out and its heart rate, respiratory rate and temperature were recorded.

Blood samples were collected into heparin-treated and plain tubes for the determination of cTn-T, cTn-I, total protein, albumin, AST, alkaline phosphatase (ALP), urea (blood urea nitrogen [BUN]), creatinine and lactate dehydrogenase (LDH). cTn-I and cTn-T were tested on test strips of the card I kit Combo test (Aboatech) and Tropt sensitive rapid assay (Roche), respectively, according to the manufacturer's instructions. Total protein, albumin, BUN, creatinine, ALP and LDH were measured using an autoanalyser (Ektachem DT System; Kodak) with commercial kits (Vitros Chemistry). Blood was also taken into EDTA-treated tubes for the determination of white blood cells, red blood cells, haemoglobin and haematocrit. The test results were considered positive when two red-brown bands appeared in the read window after 15 to 20 minutes (Fig 1). cTn-I and cTn-T were also tested in blood from a healthy calf of similar age and body condition. Soon after the collection of samples, the presented calf died.

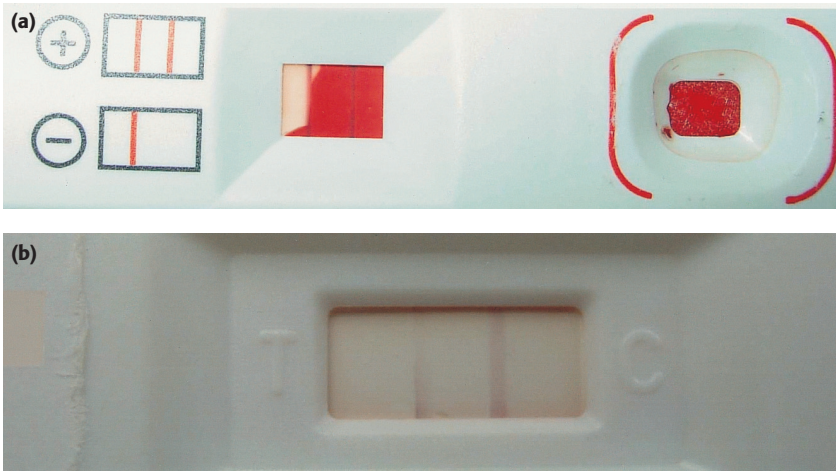


FIG 1: (a) Positive result for cardiac troponin-T in heparinised blood. (–) Control line (negative result), (+) Test line (positive result). (b) Positive result for cardiac troponin-I in serum. C Control line, T Test line

Haematological and biochemical findings in the calf were normal except for ALP, AST and LDH: a two-fold increase in serum ALP, AST and LDH concentrations was determined. cTn-I and cTn-T were positive in the case, while a negative result was obtained from the healthy (control) calf. As part of another study, 20 healthy cattle were tested, with no false positives being found (V. Gunes, unpublished data).

A postmortem examination was carried out, and heart samples were taken for histopathological examination, routinely processed and stained with haematoxylin and eosin, Von Kossa and Van Gieson stains. Macroscopically, greyish foci of irregular size in the ventricles of the heart were identified. Microscopically, lesions characterised by hyaline degeneration and necrosis, accompanied by an intense mononuclear cell infiltration, were seen on the myocardium. There was also hyperaemia, epicardial haemorrhage and oedema. Thus, the cTn-I and cTn-T kits successfully determined the presence of myocarditis.

Blood tests for the diagnosis of cardiac injury in animals are inadequate. Although troponin complex proteins have been determined in animals, there are a limited number of studies on their clinical use. Studies in animals have mainly included laboratory animals and have been designed for the detection of MI in human beings (McAuliffe and Robbins 1991, Ricchiuti and others 1998, Charles and others 2000, Fredericks and others 2001). Clinical trials have indicated that serum cardiac troponins are the first biochemical markers to appear during the course of acute coronary disease in human beings (Boccara and others 2000, Ooi and others 2000). The diagnosis of myocarditis in farm animals due to, for example, hereditary factors, vitamin E and selenium deficiency, microorganisms, traumatic injuries or heart parasites (Van Vleet and Ferrans 1986), is important because in such cases the animals could be slaughtered before sudden death. Therefore cardiac markers such as tropins could be used to diagnose myocarditis in animals.

Although no attempts were made to isolate virus, the case history and pathological findings were consistent with previous reports and suggestive of FMD (Van Vleet and Ferrans

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1986) as the disease was endemic in the region (Erdogan and others 2004).

Analysis of the calf's serum revealed considerable increases in AST and LDH, which might indicate myocardial degeneration. Although the concentration of AST increases during cellular degeneration of striated muscle and liver, increases in LDH together with AST are indicative of myocarditis (Cardinet 1997). The positive results of the cardiac troponin tests supported the results of serum enzyme analyses and pathology.

The present findings suggest that cardiac troponin tests produced for use in human beings could also be used in cattle, as the antigenic similarity of human and bovine cTn-I is 96·4 per cent (O'Brien and others 1997). The use of tests for cardiac troponins may enable veterinarians to diagnose myocardial degeneration due to FMD in cattle herds, which may lead to early intervention. Cardiac troponins are important markers for myocardial injury because they are released into the blood soon after any microinjury occurs in the myocardium and can therefore be used for the diagnosis and prognosis of cattle suffering from cardiac problems. The clinical use of the markers should be investigated in detail in the field.

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