Scientific Report

Myocardial epithelial inclusions in a Holstein calf

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Summary

Epithelial inclusions were detected in myocardium of a 2-month-old male Holstein calf. Microscopically, nonpurulent myocarditis along with focal tubular and acinar structures covered with cuboidal epithelial cells was seen in the myocardium. Most tubular and acinar structures stained with Masson's trichrome were found to be surrounded with a dense collagen. Tubular and acinar structures had PAS positive basement membrane. Immunohistochemical staining revealed that tubular and acinar structures were both vimentin and pancytokeratin positive, while the connective tissue was only vimentin positive.

Key words: Calf, Epithelial inclusion, Heart

Introduction

Myocardial epithelial inclusions have been reported as heterotrophic lesions formed during the organogenesis of the draft heart by coalescence to the developing foregut (Guarda and Biolatti, 1997; Robinson and Maxie, 1985). They have been reported in cattle, rats, swine, and human (Rabson and Thill, 1948; Jolly, 1965; Alison *et al*., 1987; Baker *et al*., 1993). Although it has previously been suggested that the lesions had endothelial, epithelial, mesothelial or endodermal origin, nowadays it is thought that they have endodermal origin (Tursi *et al*., 2009). It has been reported that bovine myocardial epithelial inclusions are most likely endodermal in origin and that they are choristomas (Baker *et al*., 1993).

Myocardial epithelial inclusions are found, incidentally, while inspecting the organs or during necropsy (Bundza and Dukes, 1978). Macroscopically, they are usually of white color, hard, plaque-like focal or multifocal masses of approximately 0.5-2 cm in diameter on the left ventricular wall (Baker *et al*., 1993; Tursi *et al*., 2009). Microscopically, they are characterized by tubular and acinar structures lined by cuboidal epithelial cells (Jolly, 1965; Nordstoga and Aleksandersen, 1988).

This paper describes the presence of a rarely seen case of the myocardial epithelial inclusions in the heart of a calf by histological and immunohistochemical methods.

Case presentation

The systemic necropsy was made on a 2-month-old male Holstein calf, which died due to a malignant form of foot and mouth disease (FMD), and tissue samples from the heart were fixed in 10% neutral formalin and embedded in paraffin. Sections were stained with haematoxylin and eosin (H&E), masson's trichrome (MTC) and periodic acid schiff (PAS). Tissue sections were also stained utilizing the avidin-biotin-peroxidase complex method immunohistochemically for pan-cytokeratin and vimentin antibodies (Bourne, 1985). Mouse anti-human vimentin and mouse anti-human pan-cytokeratin kits (Dako Corp, Carpinteria, CA) were used. The primary antibodies were diluted to 1:80

and 1:100, respectively. The skin sections were used as positive and negative controls for vimentin and pan-cytokeratin. The epidermal layer was positive control for pan-cytokeratin, while the dermal layer was positive control for vimentin. The immune complexes were stained with diaminobenzidine tetrahydrochloride (DAB) and counter-stained with Mayer's hematoxylin (M-H).

Results

Macroscopically, dark red-colored lungs with increased volume were noted. A serous fluid of approximately 20 ml volume was found in the pericardial sac. There were numerous pale foci on the transverse section of the heart. There was no change in the atrioventricular node region or on the atrial walls.

In the myocardium, near the endocardium of the left ventricle, tubular and acinar structures with focal location, lined with cuboidal epithelium were noted microscopically (Fig. 1) with accompanying non-purulent myocarditis characterized by myofibrillar necrosis, interstitial edema, and lymphohistiocytic infiltrations. The cells forming these structures had eosinophilic cytoplasm, with uniform and round-to-oval nuclei. They did not show considerable mitosis or atypia. Tubular structures were of similar size, but some branched into anastomosing channels. Myofiber associated with lymphohistiocytic cell infiltrations were seen among these structures (Fig. 2). The lumens of the tubular structures were usually empty or contained an eosinophilic, globular, amorphous material. Most of the tubular and acinar structures were surrounded by dense collagen stained with MTC. Tubular and acinar structures had a PAS-positive basal membrane. The tubular epithelium was pan-cytokeratin-positive (Fig. 3). The connective tissue and the tubular structures were strongly positive for vimentin (Fig. 4).

Discussion

Macroscopically, although there is no specific anatomical location for myocardial epithelial inclusions in cattle, these lesions have usually been detected on the left ventricular wall and less commonly on the apex of the heart and the interventricular septum (Jolly, 1965; Bundza and Dukes, 1978; Baker *et al*., 1993). Some authors have defined these lesions as cysts on the atrial wall or the base of the heart and they

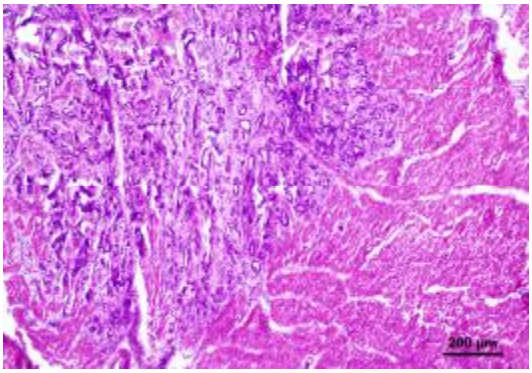

Fig. 1: Tubular and acinar structures in the myocardium, (H&E, Bar 200 µm)

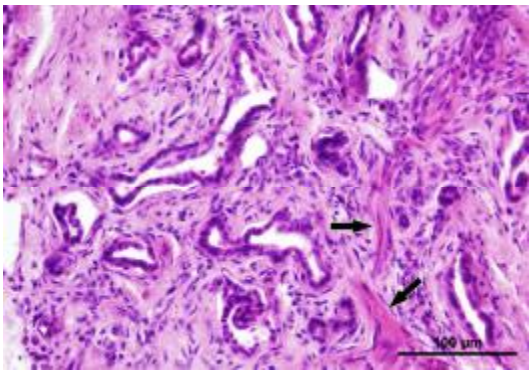

Fig. 2: Appearance of myofiber (arrows) and lymphohistiocytic cell infiltrations among tubular and acinar structures, (H&E, Bar 100 µm)

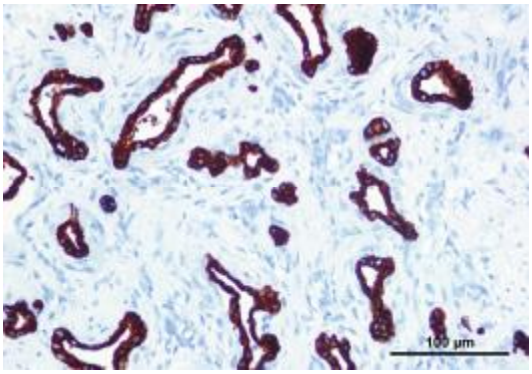

Fig. 3: Cuboidal epithelial cells of tubular structures were positive for pancytokeratin, (Bar 100 µm)

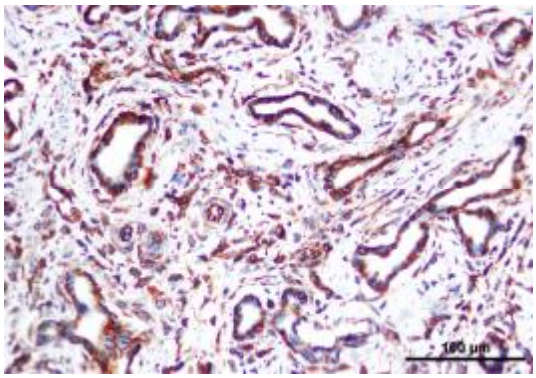

Fig. 4: Connective tissue and tubuls covered by cuboidal epithelial cells were positive for vimentin, (Bar 100 µm)

have suggested that these could represent ectopic thyroid tissue. Ectopic thyroid tissue is rare in animals, except in dogs, and it is found within the adipose tissue of the intrapericardial aorta (Robinson and Maxie, 1985). In the presented case, the lesions in the heart of the calf were defined both microscopically and immunohisto-chemically; however, the changes could not be differentiated from the lesions of FMD, macroscopically. In addition, it has been reported that macroscopic changes were similar to those with degenerated *Taenia saginata* cysts, granuloma, lymphoid infiltrations or tumor metastasis (Nordstoga and Aleksandersen, 1988). Histologically, they can be misdiagnosed as tumors such as metastatic adenocarcinoma or intracardiac mesothelioma (Shields and Popp, 1979). Microscopically, these lesions are non-encapsulated, and composed of tubular or acinar structures surrounded by collagen of varying density. The structures are mostly lined with cuboidal or columnar or less commonly squamous or transitional epithelial cells (Nordstoga and Aleksandersen, 1988; Baker *et al*., 1993). Our case had similar microscopic changes.

The origin of myocardial epithelial inclusions can be distinguished by immunohistochemistry, and all epithelial types with endodermal and ectodermal origin are positive for cytokeratin as for mesothelial cells (Nordstoga and Aleksandersen, 1988; Baker *et al*., 1993). Epithelial inclusion cells in bovines, were positive with cytokeratin (Baker *et al*., 1993; Tursi *et al*., 2009). Although we determined positivity for pan-cytokeratin; it has been noted in a previous study that this is not a helpful criterion to find the origin of the epithelial inclusions in cattle (Baker *et al*., 1993). Vimentin positivity observed in the present study was consistent with the findings of previous studies. Simultaneous expression of vimentin and cytokeratin is not unusual in some endodermally-derived carcinomas, in the fibrous component of mesotheliomas, some sarcomas, the normal mesothelium, thyroid epithelium, granulosa cells, and the endometrium (Baker *et al*., 1993). Our immunohistochemical findings suggest that myocardial epithelial cells have either endodermal or mesothelial origin.

In our case, myofiber and severe lymphohistiocytic infiltrations were found in the stroma. The infiltrations were thought to be related to the non-purulent myocarditis due to the FMD. However, there has been no mention of the presence of myofiber within the connective tissue among tubular structures. It has been suggested that myocardial epithelial inclusions are congenital remnants of endodermal tissue misplaced during organogenesis and non-proliferate, represent metastatic growths or malignant transformation (Baker *et al*., 1993). The fact that myocardial epithelial inclusions are seen in cattle of all age groups, and the similarity in size and anatomical location of these lesions is evidence of this hypothesis. In this case, the lesion on the left ventricular wall is consistent with the above-mentioned hypothesis. It has been emphasized that these masses resemble malignant myocardial mesotheliomas in rats, but their biological behavior is benign (Goodall *et al*., 1975; Alison *et al*., 1987). Similarly, the epithelial inclusions in cattle do not show proliferation or malignancy; however, these lesions may develop a malignant characteristic (Baker *et al*., 1993). The presence of myofiber among the tubular structures seen in this case suggests the possibility of proliferation of these structures.

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