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SPONTANEOUSLY ARISING DISEASE

Pathological Features of Systemic Necrotizing Vasculitis (Polyarteritis Nodosa) in Sheep

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Summary

Polyarteritis nodosa (PAN) is a systemic vasculitis of unknown aetiology affecting small- and medium-sized arteries of multiple organ systems without involvement of pulmonary arteries. This report describes four cases of PAN in sheep from different flocks. Three of these animals displayed clinical signs of locomotor disturbance. Gross necropsy findings included bilateral nodular thickening of vessels together with thromboses and aneurysms at several locations. Microscopically, small- to medium-sized arteries of the kidneys, ovary, uterus and skin were consistently involved and other locations were affected less frequently. Arteries within the lung were normal in all animals. Vascular lesions were characterized by focal fibrinoid necrosis, rupture of the internal elastic lamina and transmural infiltration of mononuclear inflammatory cells, extending into the perivascular tissue. In the kidney, many arteries showed narrowing or occlusion of the lumen and marked fibrosis. The distribution of arterial lesions was segmental, showing alternation of affected and microscopically normal areas. Immunohistochemical studies did not identify immune complex deposition. The inflammatory infiltrates were composed of T lymphocytes and macrophages, suggesting that a cell-mediated immune response may be involved in the pathogenesis of this disease.

Keywords: immunohistochemistry; polyarteritis nodosa; sheep; systemic vasculitis

Introduction

Polyarteritis nodosa (PAN) is a systemic vasculitis of unknown aetiology affecting small- and medium-sized arteries of multiple organ systems, particularly the kidneys, heart, liver and gastrointestinal tract (Bert *et al*., 1999; Schoen, 2005). PAN is well recognized in man and is most often diagnosed in young adults (Klusmann *et al*., 2006), but may also occur in adults (Fourcade *et al*., 2005) and children (Bert *et al*., 1999; Kutlu *et al*., 2004).

In man, classical systemic PAN is often associated with hepatitis B viral infection (Cohen and Guillevin, 2004; Maxie and Robinson, 2007), but localized cutaneous PAN, mainly affecting the lower limbs (Bravi and Martín, 2003) and associated with lymphoedema (Vignes *et al*., 2005), has been also described. Cutaneous PAN has been related to streptococcal infection (Albornoz *et al*., 1998), Crohn's disease (Komatsuda *et al*., 2008) and hepatitis C virus infection (Soufir *et al*., 1999).

Human PAN is characterized by nodular thickening of the walls of medium and small muscular arteries and microscopical lesions are always segmental, with destruction of the vessel wall architecture followed by the formation of aneurysms and thromboses at the site of the lesion (Guillevin, 2002).

In domestic animals this disease has been sporadically reported in sows (Hamir, 1980; Liu *et al*., 2005), cattle (Filippich and Mudie, 1972), dogs with canine juvenile polyarteritis syndrome (CJPS) (Snyder *et al*., 1995) and cats (Altera and Bonasch, 1966). Only two cases of PAN have been reported in sheep (Helmbolt *et al*., 1959; Landsverk and Bratberg, 1979) and several cases of meningeal segmental polyarteritis of unknown aetiology have

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been described (Schock *et al.*, 2009). In domestic and wild animals, PAN has been correlated with protozoan (*Sarcocystis* spp. in sheep or encephalitozoonosis in blue foxes), viral (aleutian disease in mink) and or bacterial (erysipelas in swine) infections (Nordstoga, 1976; Landsverk and Bratberg, 1979), but toxic agents and genetic, autoimmune and allergic factors may be also implicated (Walvoort *et al.*, 1987).

In man, systemic necrotizing vasculitides related to hepatitis B virus infection are thought to be triggered by the formation of immune complexes. However, in classic PAN immune complexes are absent and it has been shown that the cellular component of the lesions is mainly formed by dendritic cells and $CD4^+$ T lymphocytes, suggesting the involvement of a cell-mediated immune response (Porter *et al.*, 2003; Hernández-Rodríguez and Cid, 2008).

The present report describes the pathological and immunohistochemical features of PAN in four sheep.

Materials and Methods

Animals

Four female sheep of the Assaf breed, from four different flocks dedicated to milk production, were the subjects of this study. There was no relationship between the flocks and they were located in three different areas, with distances between them ranging from 100 to 183 km, all within the Castilla y León region, in the northwest of Spain. Sheep numbers 3 and 4 were from different flocks in the same area. The animals were submitted to the Pathology Diagnostic Service of the Veterinary Faculty of León over an 8-year period (2000–2008). Clinical information was supplied by the referring veterinary practitioners or by direct observation of the animals.

Pathological Examination

Sheep 1 and 4 were submitted alive and a complete necropsy examination was performed after intravenous injection of a lethal dose of barbiturate (sodium thiopental). Sheep 2 was received dead for necropsy examination and sheep 3 was humanely destroyed on the farm and after post-mortem examination, tissue specimens were submitted by the veterinary practitioner.

Tissue samples were collected from the kidneys, ovary, uterus, skin, liver, gallbladder and lungs. Samples of skeletal muscle, encephalon, spinal cord (sheep 1, 2 and 4) and heart, spleen, adrenal gland and mammary gland (sheep 2 and 4) were also collected. Tissue samples were fixed in 10% neutral buffered formalin and embedded in paraffin wax. Sections (4 μm) were stained with haematoxylin and eosin (HE), orcein–Masson Goldner stain, periodic acid–Schiff (PAS) and Perls' Prussian blue.

Immunohistochemistry

Selected sections with lesions compatible with PAN were immunolabelled using the avidin–biotin–peroxidase complex (ABC) method (Vectastain Standard®; Vector Laboratories, Burlingame, California, USA), according to the manufacturer's instructions. Primary antibodies were specific for T lymphocytes (rabbit anti-human CD3 polyclonal antibody; 1 in 100 dilution; Dako, Glostrup, Denmark), B lymphocytes (mouse anti-human CD79 αcy monoclonal antibody; 1 in 50 dilution; Dako), macrophages (VPM32 mouse anti-ovine monoclonal antibody; 1 in 200 dilution; Department of Veterinary Pathology, University of Edinburgh, Edinburgh, UK, and mouse anti-human CD68 monoclonal antibody; 1 in 100 dilution; Serotec Ltd., Oxford, UK) and IgG (rabbit anti-goat IgG polyclonal antibody; 1 in 1,000 dilution; Eivai Bios Laboratories, Horsham, UK). Antigens were unmasked by heat treatment by either boiling the slides for 10 min in 0.1 M citrate buffer pH 6.0 (for CD3 and CD68) or by placing them in a microwave at 400 W for 20 min (for CD79 αcy). Trypsin digestion was applied for the detection of IgG. The reaction was developed with 3,3′-diaminobenzidine tetrahydrochloride (DAB substrate kit for peroxidase; Vector Laboratories) and sections were counterstained with Mayer's haematoxylin. Sections from normal ovine lymph nodes were included as positive controls in each reaction. The specificity of the technique was controlled by omission of the primary antibody and also by its substitution with either normal mouse or rabbit serum.

Results

Clinical Findings

Three of the sheep (1–3) were referred with signs of muscular pain, subcutaneous swellings and progressive hindlimb weakness to the extent that they were unable to walk. Sheep 4 showed progressive weight loss.

Gross Lesions

Sheep 1–3 showed subcutaneous oedema of both hindlimbs with nodular thickenings in the saphenous, tibial and plantar arteries (Fig. 1a). Erythematous areas of ulcerated skin, petechial haemorrhages in the flank skin and perineal subcutaneous oedema were also noted in sheep 2.