Pathology in Practice

History

During 3 consecutive breeding seasons, a number of lambs had died shortly after birth in a flock of Sarda sheep in Sardinia. Affected lambs had hairless areas of skin on the limbs and pinnae; the hooves were absent in 1 lamb. The flock included approximately 300 ewes and 4 rams, and records indicated that 10 lambs were affected with this congenital skin disorder within the preceding year. Flock feeding relied on the exploitation of natural pastures; the diet was supplemented with concentrate and hay during the milking period. The owner reported that no new introduction of sheep had occurred before the onset of the first clinical case. A dead female lamb was transported to the diagnostic laboratories of the Istituto Zooprofilattico Sperimentale della Sardegna for postmortem examination and subsequent histologic and immunohistochemical (IHC) investigations.

Clinical and Gross Findings

The lamb had been found dead at the farm 2 hours after birth. Macroscopically, there were symmetric, reddish, translucent, and well-demarcated alopecic skin areas on the pinnae and the more distal portions of the forelimbs (Figure 1). No additional lesions involving other organs were identified during necropsy. On the basis of morphologic characteristics, the fetal development of the lamb appeared normal.

Formulate differential diagnoses from the history, clinical findings, and Figure 1—then turn the page —
Histopathologic Findings

Samples of both macroscopically affected and apparently normal skin were collected from several body regions of the lamb. Additional tissue samples of major organs such as heart, lungs, liver, kidneys, and brain were also collected. These samples were subsequently processed for histologic examination by means of paraffin embedding, microtome sectioning at a thickness of 5 µm, and final staining with H&E or periodic acid-Schiff stain. Finally, DNA was isolated from liver tissue for genetic analyses of the LAMA3 gene, which encodes for 2 laminin subunits.

Microscopically, the damaged skin areas lacked epidermis, including basement membrane, as well as adnexal structures (hair follicles, sebaceous glands, and sweat glands). At the junction between unaffected skin and affected tissue, the epidermis ended abruptly, with its margins remaining strongly adherent to the underlying dermis, with a prominent inflammatory infiltration by neutrophils and lymphocytes. In the skin adjacent to the damaged areas as well as in other body regions, the epidermis was morphologically normal and adherent to the dermis, even after injury was inflicted to test the resistance. Bullae or dermoeipidermal clefting was not evident in any cutaneous area.

These skin lesions were further characterized by means of IHC staining to detect cytokeratin. The IHC staining was performed with a routine biotin-streptavidin method, which included a mouse monoclonal primary antibody (final dilution, 1:50) against antigenic determinants of most cytokeratins in subfamilies A and B (which are both expressed in normal ovine skin), use of 3,3'-diaminobenzidine tetrahydrochloride as the chromogen, and Meyer hematoxylin counterstaining. Results of the IHC analysis confirmed the complete absence of epidermis and related adnexal structures from all affected skin areas (Figure 2). In contrast, all regions of unaffected skin had an IHC staining pattern expected for normal skin. The preliminary data from the genetic investigations indicated that there were no point mutations or deletions in the LAMA3 gene that could be considered to be associated with the dermal changes in the lamb.

Morphologic Diagnosis and Case Summary

Morphologic diagnosis: congenital, focal aplasia of the epidermis and adnexal structures, associated with superficial necrosis and extensive dermal inflammation.

Case summary: epitheliogenesis imperfecta (EI) in a lamb.

Comments

Sarda is the sole breed comprising the large sheep population of Sardinia, Italy. In this breed of mediumsized dairy sheep, the mean body weight of ewes is 50 kg (110 lb) and that of rams is 70 kg (154 lb). The lambing period extends from November to February.

On the basis of history, clinical signs, histopathologic changes, and IHC analysis results, a final diagnosis of EI was made for the lamb of this report. Epitheliogenesis im-
A detailed histopathologic description of 70 cases of EI in piglets has been published. Of the 70 affected piglets, 65 (93%) were males, suggesting that EI could be a sex-related condition in swine. Nevertheless, currently available data for sheep are scarce and do not support any influence of sex on EI occurrence in lambs. Additionally, in the swine study, the morphologic features of the hair follicles at the junction of normal and affected skin were variable, allowing definition of different histopathologic patterns, namely areas in which follicles were apparently normal, multifocally increased in number or size, or dysplastic. Although similar changes have not been categorized in lambs because of the low number of cases available for investigation, the histopathologic pattern in the affected lamb of the present report can be considered normal on the basis of the pattern definitions for affected swine.

Interestingly, a partial absence of dermis was further observed in piglets; thus, aplasia cutis (the term used in human medicine) may be a more appropriate definition of this condition. In this respect, it should be also underscored that EI, aplasia cutis, and epidermolysis bullosa (a neonatal genodermatosis also affecting lambs) have been commonly used as synonyms, thereby generating some confusion. In the lamb of the present report, considering that the congenital disorder affected only the epidermis, it may be correctly defined as EI.

Absence of adnexal structures that is restricted to the diseased skin regions is another interesting morphologic hallmark of EI. On the basis of histologic observations in the case described in this report, we believe that the absence of the adnexal structures was not due to an inflammatory process affecting the damaged skin; conversely, the absence of the adnexal structures may have represented an expected consequence of deficient epithelial development and morphogenesis during embryogenesis. Indeed, results of a study of mice homozygous for a disrupted p63 gene (a homologue of the tumor suppressor p53) indicated that congenital defects of squamous epithelial development in the skin may be associated with absence of the epithelial appendages.

Infection, vascular malformations, and defective angiogenesis, and various teratogens have been proposed as causative factors of aplasia cutis in humans, but no unifying theory has been identified. Epitheliogenesis imperfecta probably represents a simple autosomal recessive trait in cattle, horses, swine, and sheep. The occurrence of EI in the flock of the present report was not associated with the introduction of new animals. In this flock, however, these data were not sufficient to rule out the presence of a genetic causative factor. Further investigations are needed to better characterize the possible association of this birth defect with 1 or more unknown genetic factors. Indeed, EI appears to be a recessively inherited trait in piglets.

Epidermolysis bullosa is often misdiagnosed as EI. Epidermolysis bullosa includes a group of hereditary mechanobullous diseases characterized by cutaneous blisters in response to trauma. This disease in sheep has been repeatedly reported, and definitive diagnosis is made on the basis of history and physical examination and histopathologic findings. The histopathologic findings are characterized by subepidermal vesicles, resulting from dermoepidermal separation below the basement membrane, that are present at birth or develop within a few days or weeks after birth. Furthermore, epidermolysis bullosa has been associated, both in horses and sheep, with a partial deletion of LAMA3 and LAMC2 genes that encode for 2 laminin subunits. Laminin is one of the most important components of the basement membrane of the epidermis and acts as a pathway for the migration, differentiation, and organization of cutaneous epithelial cells. Nevertheless, results of LAMA3 sequencing for the lamb of the present report did not suggest an involvement of this gene in the pathogenesis of EI.

References