First clinicopathological characterization of body wrinkle condition in Merino sheep and its association with Orf virus infection

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Abstract

Merino sheep exhibit a high follicular density, enhancing wool yield but predisposing them to inherited cutaneous disorders, such as breech wrinkle, which is characterized by the appearance of multiple skin folds in the breech area. This study provides the first clinicopathological description of the so-called body wrinkle, a congenital skin condition in Merino lambs where the folds affect the whole-body skin. Four affected lambs displayed generalized alopecia with excessive skin folds. Hematological analysis showed neutrophilia, lymphopenia, and eosinopenia. Histological examination revealed follicular dysplasia, follicular keratinization and keratosis, and degeneration of follicular epithelium, particularly in the outer root sheath. The information obtained from pedigree analysis suggested an autosomal recessive inheritance pattern. All affected lambs exhibited severe OrfV lesions in the oral cavity and muzzle, persisting from 2 weeks of age until death at 1–2 months. Our results suggest that body wrinkle condition might favor a more prolonged clinical course and more severe outcome of OrfV infection in lambs. Further genetic and immunopathological investigations are needed.

Keywords: Body wrinkle condition, hair follicle, lamb, Orf virus, skin

Merino sheep exhibit a higher density of hair follicles compared to other breeds, resulting in a greater number of wool fibers per unit area of skin and their characteristic fine, soft fleece.[13, 19] However, this can be associated with undesirable abnormalities, including the breech wrinkle condition,[11] an inherited trait characterized by excessive skin folds in the breech area. When these skin folds affect the entire body surface, this condition is referred to as body wrinkle. Even if a larger number of wrinkles may be a desirable trait to increase wool yield, they pose considerable challenges to both animal welfare and production systems.[20] A classification based on five categories ranging from no wrinkles (smooth skin, score 1) to extreme wrinkles (extensive with deep folds, score 2 to 5) is currently used.[4] Mild and moderate wrinkles present intermediate risks, while severe and extreme wrinkles can create microenvironments conducive to the accumulation of dirt, feces, and urine, increasing susceptibility to cutaneous myiasis (breech flystrike), often co-existing with secondary infections.[12] This cutaneous condition in Merino lambs may also predispose animals to viral skin diseases, including contagious ecthyma (CE) caused by the Orf virus (OrfV), a member of the *Poxviridae* family.[8] CE is a globally distributed, highly contagious zoonotic mucocutaneous disease that primarily affects young small ruminants.[10] OrfV infects and replicates within proliferating epithelial cells of the epidermis, as well as the oral and gastrointestinal mucosa. Consequently, pre-existing wounds or mucocutaneous defects significantly facilitate viral infection.[2,16] Immune evasion mechanisms,[5] prolonged environmental stability of infectious viral particles,[21] and the existence of asymptomatic-infected carrier animals play also a role in the persistence of OrfV in affected farms.[18] CE typically manifests as papules, vesicles, and pustules that progress into scabby proliferative lesions. These lesions commonly affect the lips, lip commissures, muzzle, periocular region, eyelids, ears, forelimbs, groin, and udders. Frequently, multifocal erosions and ulcers can be observed in the oral mucosa, esophagus, and rumen.[14]

Understanding the pathological implications of the body wrinkle condition is crucial for improving welfare in Merino sheep. This study provides a comprehensive clinicopathological characterization of body wrinkle-affected lambs, providing the first detailed histological description, highlighting its likely influence on the progression of OrfV infection. This work also suggests a possible inheritance pattern for this condition.

On a semi-intensive farm including 700 Merino ewes, about 2% of lambs presented annually with congenital, progressive, generalized alopecia and wrinkled skin, characterized by multiple pronounced folds. These animals also exhibited multiple erosions and ulcers of the oral and nasolabial mucosa at two weeks of age, subsequently developing into severe, multifocal, scabby proliferative dermatitis on the muzzle. These lesions were clinically compatible with

OrfV infection. These lesions impaired the lamb's ability to suckle, causing weakness, poor body condition, and leading to death at 1–2 months of age. The mortality rate of affected lambs was 100%. Some lambs that lacked the body wrinkle condition also suffered from oral and nasolabial mucosal OrfV-associated lesions that resolved spontaneously within 6 weeks.

Four affected lambs (three males and one female), born to different unaffected ewes, were studied in the ruminant clinical service (SCRUM) at the Veterinary Faculty of the University of Zaragoza. Three of the parturitions were singleton deliveries, whereas one was a twin delivery producing one lamb with body wrinkle cutaneous lesions, whereas the co-twin was clinically normal. Physical examinations were performed and photographs obtained weekly from birth over the course of 1-2 months, until natural death occurred. Skin biopsies from the neck, trunk, and breech zone were collected weekly for histological evaluation. Biopsies from three healthy Merino lambs of the same age served as negative controls. Whole blood samples were collected from affected lambs and their ewes in EDTA-containing tubes. These samples were used for hematological analysis using IDEXX ProcyteDx automatic hematology counter (IDEXX laboratories, Westbrook, ME, USA). Measured parameters included leukocytes (K/mL), erythrocytes (M/µL), hemoglobin (g/dL), hematocrit (%), platelets (K/µL), mean corpuscular volume (fL), mean corpuscular hemoglobin (pg), mean corpuscular hemoglobin concentration (g/dL), and reticulocytes (K/µL). White blood cells were also evaluated by counting neutrophils, lymphocytes, monocytes, basophils, and eosinophils (K/µL). Affected lambs died between one and two months of age, following progressive clinical deterioration. Necropsies were performed and skin samples from the muzzle, lips, neck, trunk, and breech region; lung (five lobes); mediastinal, prescapular, and retropharyngeal lymph nodes; thymus; spleen; and central nervous system were collected for histological analysis. Sections of the muzzle and lips exhibiting OrfV-associated lesions and blood samples from lambs and their ewes were subjected to PCR, targeting the OrfV *045* gene and OrfV *B2L* gene.[9, 14, 15]

At 2-3 weeks of age, the four affected lambs exhibited significant clinicopathological findings consisting of poor body condition and delayed growth. They also presented acute, severe, multifocal erosions and ulcers of the palate and tongue. Over the following weeks, the lambs progressively lost hair starting from the breech area to their entire body, exposing generalized, prominent skin folds (Fig. 1a, b). By this stage, they also developed severe, multifocal scabby proliferative dermatitis affecting the muzzle and lip commissures, occasionally with multifocal pustules (Fig. 1c, d). No other lesions were observed during postmortem examinations.

Microscopically, control lambs exhibited a normal dermal architecture with abundant adnexa and numerous, well-organized sebaceous glands surrounding hair follicles (Fig. 2a). In contrast, affected lambs displayed increased numbers of follicular complexes with tortuous shapes and marked architectural disorganization (Fig. 2b). Primary and secondary follicular lumina were dilated and had prominent keratosis. Hypertrophy and hyperplasia of outer root sheath epithelial cells resulted in thickening of the follicular infundibulum and external isthmus (Fig. 2c). Some of these epithelial cells showed cytoplasmic swelling consistent with hydropic degeneration (Fig. 2c). Despite these changes, the follicular cycle remained intact, predominantly in the anagen phase, with appropriate hair shaft formation (Supplemental Figure S1). Sebaceous glands were markedly reduced in number and atrophic (Fig. 2c). The epidermis exhibited diffuse, mild hyperplasia with desquamative orthokeratotic hyperkeratosis extending into the follicular infundibulum.

In the tongue and palate, the mucosa had multifocal epithelial hyperplasia with numerous intraepithelial pustules. Many pustules were ruptured, forming serocellular crusts. Adjacent keratinocytes were swollen with pale eosinophilic to clear vacuolated cytoplasm, indicative of hydropic degeneration, and some contained 5–10 µm diameter, round, eosinophilic intracytoplasmic viral inclusion bodies consistent with OrfV infection. The underlying dermis contained increased numbers of small-caliber blood vessels with hypertrophic reactive endothelium. Multiple colonies of basophilic bacilli and cocci were present on the mucosal surface and embedded within serocellular crusts.

Lesions in the muzzle and lip commissures consisted of multifocal epidermal acanthosis with pronounced parakeratotic and orthokeratotic hyperkeratosis (Fig. 2d). Keratinocytes in the stratum spinosum were swollen, sometimes rupturing to form vesicles, and frequently harbored eosinophilic intracytoplasmic viral inclusions (Fig. 2d). Follicular epithelium was similarly affected. Neutrophilic exocytosis into the superficial epidermis produced intraepidermal pustules, while areas of erosion were covered by thick serocellular crusts containing numerous mixed bacteria. The dermis exhibited increased numbers of dilated, large-caliber blood vessels and lymphatics separated by clear spaces, compatible with edema and fibrin accumulation. Moderate perivascular and diffuse superficial infiltrates of neutrophils, histiocytes, and lymphocytes were also present (Fig. 2d).

Hematological analysis revealed neutrophilia (7.1; reference interval (RI) 1.17 –6.11 K/µL), lymphopenia (1.8; RI 2,54 - 9,60 K/µL) and eosinopenia (0.01; RI 0,05 -0,95 K/µL). OrfV DNA

was detected by PCR in proliferative and ulcerative lesions although it was not detected in whole blood samples.

To determine the possible inheritance model, blood samples from the four affected lambs, the ewes, and all rams present in the herd underwent a pedigree analysis based on 19 microsatellite genetic markers (AE129, CP49. CSRD24, FcB20, HSC, ILST005, ILST008, ILST011, INRA006, INRA023, INRA049, INRA063, INRA132, INRA172, MAF214, MAF65, MCM042, SPS113 and SPS115; Supplemental Materials). The identity of the ewes registered to produce the affected newborns was confirmed, and a single ram was identified to have sired three affected lambs. Results indicated that the ram siring the fourth body wrinkle diseased individual was not present in the herd at the moment of sampling, perhaps due to previous removal from the flock. One of the affected lambs had been born in a double birth. In this case, microsatellites confirmed that both newborns were fraternal twins (not identical twins), as one of them was affected while the other one remained normal.

This study represents the first histopathological description of the body wrinkle condition, a congenital skin disorder in Merino lambs, and its association with a delayed clinical outcome following OrfV infection. Breech wrinkle condition has been extensively studied macroscopically to classify Merino sheep into different categories and exclude severely wrinkled animals from genetic selection programs.[12,20] The histopathological features in this study included, among others, marked follicular dysplasia, keratinization, and keratosis together with degeneration of follicular epithelium, particularly in the outer root sheath. These alterations might explain the generalized alopecia associated with the body wrinkle condition. The follicular damage may derive from a genetic defect. Indeed, results from the parentage analysis are compatible with a possible autosomal recessive inheritance for this pathology, so that affected lambs could be homozygous for a particular unidentified mutation, carried in heterozygosis by both parents. Results on the fraternal twins support this idea; only one of them showed the undesired character. Microsatellite analysis demonstrated that even if both of them develop along the same pregnancy and shared an *in utero* environmental conditions, they carried different genetic information. If the condition is indeed inherited as a monogenic autosomal trait (and provided that suitable samples for genomic DNA isolation were biobanked), it should be possible to determine the chromosomal localization of the causal gene by genome-wide association study and/or linkage mapping and to identify the causal genetic variant by whole-genome sequencing.[3]

The gross lesions associated with this condition are highly characteristic and can strongly support a definitive diagnosis. However, certain differential diagnoses, such as mild cases of dermatosparaxis (Ehlers–Danlos syndrome) or even mange, should be considered at the macroscopic level. In all cases, additional analyses, including histological examination, are essential to establish a conclusive diagnosis. Wrinkle skin variants with exaggerated skin folds occur in the Shar-Pei breed of dogs, where this condition has been genetically linked to the *hyaluronan synthase 2 (HAS2)* gene.[17] Pronounced skin folds can alter the cutaneous microenvironment, promoting secondary bacterial and fungal infections,[7] modifying local immune responses, and influencing gene expression profiles.[1,6] Neutrophilia, lymphopenia, and eosinopenia observed in affected lambs are probably caused by secondary concomitant infections. Interestingly, a region lying in a gene defect on chromosome 9 downstream from the *ZHX2* gene (*zinc fingers and homeoboxes 2*) and upstream of the *HAS2* gene (*hyaluronan synthase 2*) has been identified to be involved in variability of breech wrinkling in the Merino breed.[3]

We propose that severe follicular lesions may result from a combination of a genetic defect and, perhaps, microenvironmental changes. The absence of histopathological changes in lymphoid tissues suggests that the pathological process is restricted to the skin. However, further research is necessary to definitively confirm the genetic basis of the body wrinkle condition and to investigate whether immune compromise contributes to its pathogenesis. This cutaneous defect possibly predisposes lambs to OrfV infection and disease progression. Whilst OrfV-associated lesions typically resolve within 3–6 weeks in lambs not affected by body wrinkle[16] (as observed in the present study), Orf lesions in body wrinkle-affected lambs persisted until death. Moreover, although OrfV occasionally causes pseudotumoral skin lesions in lambs,[18] all body wrinkle-affected lambs in this study developed proliferative pyogenic granulomas, atypical papilloma-like lesions, and pronounced angiomatous lesions. These findings may indicate that this severe skin defect could facilitate OrfV infection and progression. A stress-related impairment of the immune response that facilitates the development of these exuberant Orf lesions can be hypothesized; however, further studies are needed to investigate the potential mechanisms that may increase OrfV susceptibility in animals affected by body wrinkle.

In conclusion, this study highlights the clinicopathological changes associated with body wrinkle condition in Merino lambs and suggests that this inherited skin defect may facilitate a more prolonged and severe clinical outcomes of OrfV infection.

Acknowledgments

We are grateful to Jaume Alomar from the Servei de Diagnòstic de Patologia Veterinària, Departament de Sanitat i Anatomia Animals, Universitat Autònoma de Barcelona, for his assistance in the interpretation of the pathological results.

Declaration of Conflicting Interests

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Ethical statement

Experimental procedures were approved by the Ethical Committee of the University of Zaragoza (ref. PI10/25-Unizar) and fulfilled the requirements of the Spanish Policy for Animal Protection (R.D. 53/2013) that adopted the European Union Directive 2010/63 on the protection of experimental animals.

Funding

This research was partially supported by funding from the Aragon Government (AR15_23R) and the European Social Fund.

Author´s contributions

AG, PW and DL designed and performed the experiments; PQ, MRA, RR, EP, SVS, and LL contributed to the experimental design; AG and LL performed the pathologic evaluations; MRA, PQ, SVS and DL performed the clinical and hematological analysis; IM and RR performed the molecular analysis; the manuscript was written by AG, LL, PW and DL with contributions from the other authors.

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Figure legends

Figure 1. One-month-old Merino lamb with body wrinkle condition. a) Multifocal to coalescing alopecia revealing multiple, prominent skin folds over the trunk and flank. b) Close-up of the skin folds, showing a cerebroid pattern. c, d) Muzzle and lips with ORFV-associated lesions, characterized by focally extensive, scabby, proliferative, and necrotizing dermatitis and cheilitis (arrow).

Figure 2. Histological sections from a one-month-old healthy Merino lamb and an age-matched affected lamb with body wrinkle condition. Hematoxylin and eosin. a) Haired skin from the healthy lamb with an ordered arrangement of adnexal structures. b) Haired skin from the affected lamb showing that all follicular complexes are altered in size, shape, and position, with marked luminal hyperkeratosis (arrows). c) Haired skin from the affected lamb. Follicular epithelial cells of the outer root sheath are hypertrophic and hyperplastic, some presenting hydropic degeneration (thick arrows). Most of the sebaceous glands are atrophic (arrowhead). Follicular lumina have prominent hyperkeratosis (thin arrow). d) Lip, ORFV-associated lesions. The epidermis has severe acanthosis, with marked hydropic degeneration and microvesicles (thick arrow), and prominent parakeratotic and orthokeratotic hyperkeratosis (arrowhead), with marked hyperemia in the superficial dermis (thin arrow). Lymphocytes, neutrophils, and macrophages infiltrate the dermis. Inset: Higher magnification showing intracytoplasmic, eosinophilic inclusion bodies in the keratinocytes of the spinous layer of the epidermis.