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Veterinary Dermatology

CASE REPORT

Amplification of *Ovis aries* papillomavirus type 2 DNA from an ovine cutaneous fibropapilloma

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Abstract

Seven of 60 Perendale sheep within a flock developed single or multiple exophytic masses on their distal hind limbs. A mass was excised from one sheep and histological evaluation revealed epidermal and mesenchymal proliferation, papillomavirus-induced keratinocyte changes and marked keratohyalin clumping. *Ovis aries* papillomavirus type 2 DNA sequences were amplified using PCR.

KEYWORDS

fibropapilloma, *Ovis aries* papillomavirus, papillomaviruses, sheep, viral oncogenesis, warts

INTRODUCTION

Sheep are currently recognised to be infected by four different *Ovis aries* papillomavirus (OaPV) types. Both OaPV1 and OaPV2 were fully sequenced and deposited into GenBank by Australian researchers. While DNA sequences from both PV types have been detected in the blood of clinically normal sheep,[1] neither PV type has been associated with disease in this species. OaPV3 has been associated with cutaneous squamous cell carcinomas in sheep, while OaPV4 was detected in an ovine scrotal fibropapilloma.[2] Although not associated with any ovine disease, OaPV1 and OaPV2 DNA has been detected in bladder neoplasms in cattle,[3] and OaPV2 was reported to be the likely cause of an equine sarcoid-like mass in the mouth of a pig.[4]

Viral papillomas (warts) develop as a result of PV-induced epidermal hyperplasia. The hyperplasia is advantageous for the PV as it allows rapid PV replication and the production of numerous infectious viral particles.[5] In most species, the hyperplasia is restricted to the epidermis. However, ruminant Deltapapillomavirus types can result in hyperplasia of both the epidermis and the underlying connective tissue resulting in a fibropapilloma. Cutaneous fibropapillomas have been recognised in sheep for 50 years, yet there are only three previous reports in which molecular techniques were used to investigate the causative PV type.[2,6,7] Surprisingly, a different PV type was detected in each report with OaPV4,[2] bovine papillomavirus (BPV) type 2[6] and BPV1[7] all reported as possible causes of ovine fibropapillomas. In the present report, OaPV2 DNA sequences were amplified from an ovine skin fibropapilloma, and it is suggested that OaPV2 may be the fourth PV type associated with cutaneous fibropapillomas in sheep.

CASE REPORT

Seven of 60 Perendale sheep, at 14 months of age, developed 1–2 cm diameter, exophytic and circumscribed cutaneous masses on the cranial surfaces of their hind limbs, located approximately 5 cm proximal to the coronet. Single masses were present in five sheep with two developing either two or three lesions. The sheep had been shorn 10 weeks earlier. None of the affected animals showed other evidence of clinical disease such as lameness or visible loss of body condition.

On clinical examination, the lesions were hard and grey-to-brown with a roughened surface which variably had multiple small villous-like projections. The surrounding skin was not reddened, and the lesions were not painful. Sheep of a similar age on the same farm had developed severe crusting lesions on the feet that had caused production losses 1 year previously. Owing to concern that the current masses could be the same disease process, a mass from one sheep was surgically excised, fixed in formalin and submitted for histological examination.

Histological examination revealed a well-defined 1.5 cm diameter proliferative lesion. At the periphery, the epidermis was thickened with numerous thin rete pegs extending into underlying expanded bland fibrous tissue

(Figure 1a). Within more central areas, the thickened epidermis was arranged in broad filiform projections supported by a thick core of bland fibrous stroma (Figure 1b). The thickened epidermis throughout the mass was covered by increased quantities of eosinophilic keratin. Superficial keratinocytes had markedly expanded clear to slightly basophilic cytoplasm and keratohyalin clumping was marked throughout the lesion (Figure 1c). Aggregates of lymphocytes were present multifocally within the fibrous stroma extending into the overlying epidermis.

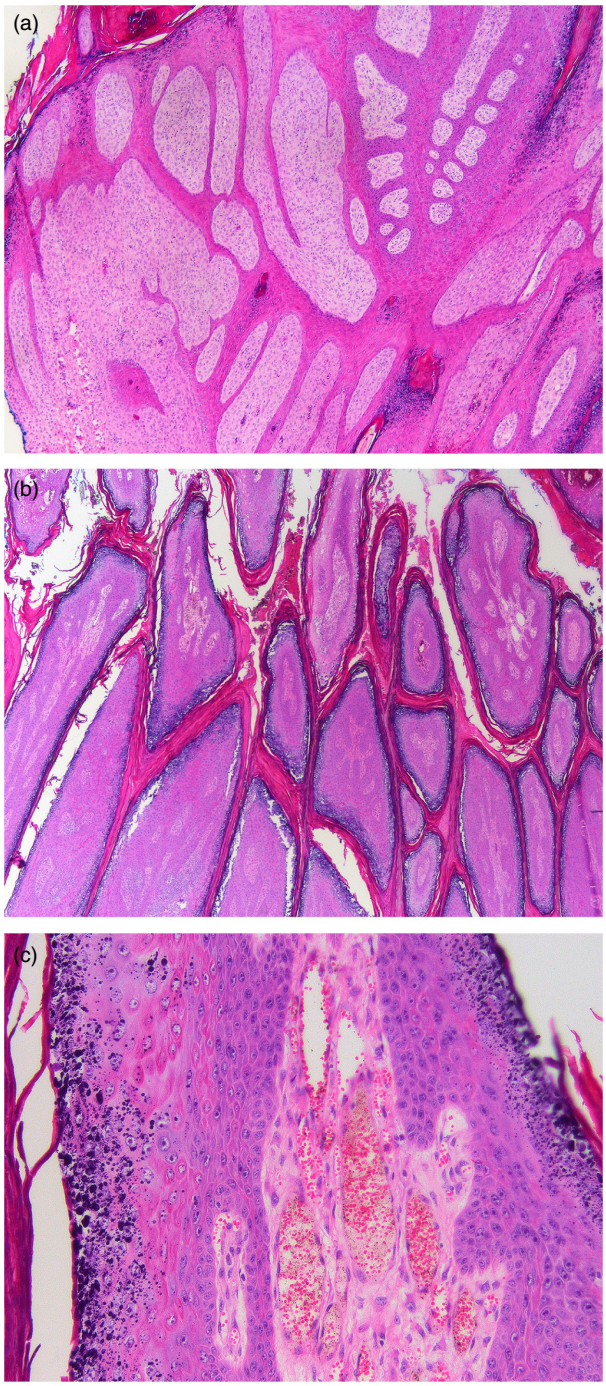

FIGURE 1 Cutaneous fibropapilloma from the distal limb of a 14-month-old Perendale sheep. (a) The periphery of the mass is a typical fibropapilloma with hyperplastic epidermis forming rete pegs that extend into underlying bland fibrous tissue. (b) Central parts of the fibropapilloma consist of filiform projections of hyperplastic epidermis supported by a core of fibrovascular tissue. Marked clumping of keratohyalin granules is visible even at low magnification. (c) The filiform projections contain a central core of bland fibrous tissue with dilated blood vessels covered by hyperplastic epidermis. Keratinocytes within the epidermis are enlarged with basophilic slightly granular cytoplasm. There is marked clumping of keratohyalin granules within the stratum granulosum.

Histological findings were consistent with a PV-induced fibropapilloma, and total DNA was extracted from a sample of the formalin-fixed paraffin-embedded tissue block. The MY09/11 and CP4/5 consensus PCR primers as well as primers specific for BPV1 and BPV2 were used to amplify PV DNA.[4,8] DNA extracted from a bovine fibropapilloma that contained BPV1 was used as a positive control for the MY09/11, CP4/5 and BPV1-specific primers, while DNA extracted from an equine sarcoid that contained BPV2 was used as a control for the BPV2-specific primers. No template DNA was added to the negative controls. DNA was amplified from the ovine fibropapilloma by both sets of consensus primers, and not by the primers specific for BPV1 or BPV2. The DNA sequences amplified by the consensus primers were compared to other sequences using the BLAST tool in the GenBank database. The 376 bp sequence amplified by the MY09/11 primers was identical to the reference OaPV2 sequence (GenBank U83595.1) while the 360 bp sequence amplified by the CP4/5 primers was 99.4% similar to OaPV2.

All lesions resolved spontaneously over the following month and no additional lesions had developed in these sheep at the time of writing.

DISCUSSION

The fibropapilloma in the present case contained histological evidence of PV infection and OaPV2 DNA sequences. While this does not prove that OaPV2 caused the lesion, the consistent amplification of OaPV2 by both consensus PCR primer sets suggests that this PV type was the most likely cause. As neither BPV1 nor BPV2 DNA was amplified by the specific primers, involvement of these PV types is unlikely.

OaPV2 is classified within the *Deltapapillomavirus* genus. As PVs within this genus typically cause fibropapillomas in their host species,[8] this provides additional support for a role of OaPV2 in fibropapilloma development in these sheep. Some ruminant Deltapapillomavirus types also are able to cause mesenchymal neoplasia in nonhost species. Evidence from the present and previous studies suggests that OaPV2, like other PVs in this genus, may cause fibropapilloma in sheep and mesenchymal neoplasia in pigs.[4]

Four studies have now used molecular techniques to investigate the aetiology of ovine fibropapillomas with four Deltapapillomavirus types—OaPV2, OaPV4, BPV1 and BPV2—identified as possible causes.[2,6,7] Hyperplastic lesions develop as a consequence of PV infection when the PV rapidly replicates and markedly increases cell replication. However, PVs typically are only able to rapidly replicate (and therefore cause hyperplastic lesions) in their own, or closely related, host species. Therefore, while OaPV2 and OaPV4 would be predicted to cause ovine fibropapillomas, sheep and cattle are not evolutionarily or genetically closely related,[9] and BPVs would not

be expected to be able to induce a hyperplastic lesion in a sheep. It is interesting that both reports of BPV-associated ovine fibropapillomas were described in sheep that are likely to have been exposed to bracken fern. Bracken fern contains immunosuppressive compounds, and it is possible that immunosuppression allowed BPVs to rapidly replicate, and so cause fibropapillomas, in a nonhost species. Alternatively, studies of papillomas in other species have shown that co-infections by multiple PV types are common.[10] Such co-infections can make it difficult to determine which PV was causative and which was present incidentally within a lesion. Co-infections can be difficult to detect without using specific PCR primers, and the use of specific PCR primers in future studies of ovine fibropapillomas may allow clarification of the causes of these lesions including potential geographical variability in the causative PV types.

A viral fibropapilloma was confirmed histologically in only one animal, yet the development of clinically similar lesions in around 10% of the sheep suggests that multiple animals developed fibropapillomas in this flock. Papillomas develop when immunologically naïve animals are exposed to a PV through microabrasions of the skin.[5] The young age of the sheep in the present report is consistent with none of these animals having being previously exposed to OaPV2. The cause of the possible outbreak in this flock is uncertain. However, skin abrasions caused by the recent shearing may have contributed to infection of multiple animals. The apparent outbreak also could have been promoted if OaPV2 had been transmitted between sheep by contaminated shearing equipment.

Compared to papillomas from cattle, horses or dogs, few ovine fibropapillomas have been evaluated histologically or using molecular techniques. This is probably a consequence of few farmers seeking veterinary attention for sheep with localised, nonpainful skin lesions that do not interfere with production. Additionally, farmers may recognise these lesions as spontaneously resolving warts. In the present case, veterinary advice was only sought as a result of the development of skin lesions in animals of similar age in the previous year. The previous lesions had interfered with production. While the possibility of the previous lesions also being papillomas cannot be excluded, other disease processes appear more likely owing to the severity of the disease.

As is observed with viral papillomas in other species,[5] all of the clinically observed lesions spontaneously resolved within 2 months of being observed. The presence of lymphocytic inflammation in the fibropapilloma that was examined histologically is consistent with spontaneous resolution already occurring in this lesion.

The most prominent histological feature of the presently described fibropapilloma was the marked keratohyalin clumping. Interestingly, this was not reported as a feature of the fibropapillomas associated with OaPV4, BPV1 or BPV2.[2,6,7] Additionally, the filiform appearance created by the hyperplastic epidermis and fibrous cores has not been reported previously. Although the role of OaPV2 in lesion development remains uncertain, evidence from the present case could suggest that fibropapillomas caused by OaPV2 may show characteristic histological features compared to other causes of ovine fibropapillomas.

AUTHOR CONTRIBUTIONS

John S. Munday: Methodology; writing – original draft. **Hanna J. Klobukowska:** Writing – review and editing; investigation. **Karen Nicholson:** Investigation; writing – review and editing.

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CONFLICT OF INTEREST STATEMENT

No conflicts of interest have been declared.

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摘要
在一个羊群中，60只Perendale绵羊中有7只的后肢远端出现了单个或多个外生肿块。从一只绵羊身上切下一个肿块，组织学评估显示表皮和间充质增殖、乳头瘤病毒诱导的角质形成细胞变化和明显的透明角质聚集。用聚合酶链式反应扩增了绵羊乳头瘤病毒2型DNA序列。

Résumé
Sept des 60 moutons Perendale d'un troupeau ont développé des masses exophytiques uniques ou multiples sur leurs membres postérieurs distaux. Une masse a été excisée sur un mouton et l'évaluation histologique a révélé une prolifération épidermique et mésenchymateuse, des modifications kératinocytaires induites par le papillomavirus et une agglutination marquée de kératohyaline. Les séquences d'ADN du papillomavirus Ovis aries de type 2 ont été amplifiées par PCR.

Zusammenfassung
Sieben von 60 Perendale Schafen in einer Herde entwickelten einzelne oder multiple exophytische Massen auf ihren distalen hinteren Extremitäten. Eine Umfangsvermehrung eines Schafes wurde chirurgisch entfernt und eine histologisch Evaluierung zeigte epidermale und mesenchymale Proliferation, Papillomavirus-induzierte Keratinozyten Veränderungen und deutliche Keratohyalinklumpung. Es wurden *Ovis aries* Papillomavirus Typ 2 DNA Sequenzen mittels PCR amplifiziert.

要約

ペレンデール種のヒツジの群れ60頭のうち7頭の後肢遠位に、単発または多発性外方増殖性腫瘤が発生した。1頭のヒツジから腫瘤を摘出し、組織学的評価を行ったところ、上皮および間葉系細胞の増殖、パピローマウイルスによるケラチノサイトの変化、顕著なケラトヒアリン顆粒凝集が認められた。Ovis aries papillomavirus type 2のDNA配列がPCR法で増幅された。

Resumo
Sete de 60 ovelhas Perendale de um rebanho desenvolveram massas exofíticas na porção distal dos seus membros posteriores. Uma massa foi removida de uma ovelha e a avaliação histopatológica revelou proliferação mesenquimal e epidérmica, alterações queratinocíticas induzidas por papilomavírus e aglomeração queratohialina. Sequências de papilomavírus *Ovis aries* tipo 2 foram amplificadas utilizando PCR.

Resumen
Siete de 60 ovejas Perendale dentro de un rebaño desarrollaron masas exofíticas únicas o múltiples en sus extremidades traseras distales. Se extirpó una masa de una oveja y la evaluación histológica reveló proliferación epidérmica y mesenquimal, cambios de queratinocitos inducidos por el virus del papiloma y marcada acumulación de queratohialina. Mediante PCR se amplificaron secuencias de DNA del virus del papiloma Ovis aries tipo 2.