Journal of the American Association for Laboratory Animal Science
Copyright 2007
by the American Association for Laboratory Animal Science

Vol 46, No 5
September 2007
Pages 69–71

Case Reports

Persistent Conjunctival Papilloma Due to Oral Papillomavirus Infection in a Rabbit in New Zealand

John S Munday,[1,*] Danielle Aberdein,[1] Richard A Squires,[2] Alfa Alfaras,[2] and Andrea M Wilson[2]

A 3-y-old female Flemish Giant pet rabbit developed a papilloma on the right nictitating membrane. Although the papilloma was excised surgically, it promptly recurred. Examination of the eye 10 wk after surgery revealed that in addition to the initial mass, 2 smaller papillomas were present on the lower eyelid. All 3 masses were excised, and histology revealed papillomatous hyperplasia of the conjunctival epithelium, koilocytosis, and intranuclear viral inclusions. Polymerase chain reaction amplified papillomaviral DNA from the largest papilloma. Sequencing of the amplicon revealed 99.3% homology with rabbit oral papillomavirus (ROPV). All 3 masses recurred after removal. In addition, the rabbit was noted to be losing weight. Weight loss continued until the rabbit died 3 mo later. All 3 papillomas persisted until death. This article provides the first description of ROPV causing conjunctival papillomas and is the first report of ROPV from the southern hemisphere. The persistence of the papillomas in this case is also unusual and may suggest that ROPV-induced conjunctival papillomas are less likely than oral papillomas to spontaneously regress. Alternatively, the death of this rabbit may indicate a compromised immune system that allowed papillomaviral persistence.

Abbreviations: CRPV, cottontail rabbit papillomavirus; PCR, polymerase chain reaction; ROPV, rabbit oral papillomavirus

A papilloma (wart) is a benign focal epithelial proliferation. Three types of papillomas have been reported to occur in domestic rabbits (*Oryctolagus cuniculus*).[9] First, rabbits develop spontaneous nonviral squamous papillomas that usually develop in haired skin.[9] Second, rabbit oral papillomavirus (ROPV) causes the development of oral papillomas,[2] usually in rabbits 2 to 18 mo of age,[12] and lesions usually regress within 60 d.[6] Third, cottontail rabbit papillomavirus (CRPV) causes cutaneous papillomas, most frequently on the eyelids and ears.[9] As many as 75% of CRPV-induced papillomas undergo malignant transformation in domestic rabbits.[4] To our knowledge, neither ROPV nor CRPV have been reported previously in rabbits in the southern hemisphere.

Case Report

A 3-y-old female Flemish Giant pet rabbit developed a 3-mm papilloma on the external surface of the nictitating membrane. The mass had first been observed 1 mo previously and had grown slowly during this time. The papilloma was excised surgically, but no analyses were performed. At 10 wk after surgery, the rabbit represented due to recurrence of the mass. In addition, 2 smaller papillomas were present on the lower eyelid close to the medial canthus. No abnormalities were noted in the oral cavity. All 3 conjunctival papillomas were removed and submitted for histology. The owner reported that the rabbit had been mated on 2 separate occasions with a pet rabbit from a different property. The last mating occurred 18 mo prior to papilloma development. Since that time, the rabbit and 1 of her progeny were housed together with no contact with other rabbits.

Histology of all 3 masses revealed well-demarcated focal epithelial hyperplasia (Figure 1). The thickened epithelium was arranged within folds that were supported by a well-developed dermal stalk. Koilocytosis, characterized by nuclear pyknosis surrounded by perinuclear vacuolation, was visible within keratinocytes in the superficial layers (Figure 2). Occasional keratinocytes contained intranuclear, spherical, eosinophilic bodies consistent with papillomaviral inclusions (Figure 3). Few inflammatory cells were present in the dermis or epidermis of the papillomas. There were no hair follicles or adnexal structures in the submitted samples, confirming that these masses had developed within the palpebral conjunctiva.

The epithelial hyperplasia, koilocytosis, and intranuclear inclusions were consistent with a viral papilloma. Polymerase chain reaction (PCR) was used to confirm papillomaviral involvement. DNA was extracted from the largest papilloma for PCR. Briefly, a 10-µm section of formalin-fixed paraffin-embedded tissue was washed with xylene and then ethanol, and DNA was extracted into 150 µL of distilled water using a DNeasy blood and tissue kit (Qiagen, Hilden, Germany) according to manufacturer's instructions. The primer set FAP59–FAP64 was used to amplify conserved regions of the papillomavirus L1 gene.[3] Final concentrations of the reaction components were 1× PCR buffer, 1.5 mM $MgCl_2$, 200 µM dNTP, 0.25 µM of each primer, 1.25 U platinum *Taq* DNA polymerase (Invitrogen Corporation, Carlsbad, CA), and 2.5 µl template DNA in a final reaction volume of 50 µl. Amplification occurred in a thermocycler (Px2, Thermo Electron Corporation, Milford, MA)

Received: 19 Mar 2007. Revision requested: 23 Apr 2007. Accepted: 26 Apr 2007.
[1]Department of Pathobiology, [2]Department of Infectious Diseases, Institute of Veterinary, Animal and Biomedical Sciences, Massey University, Palmerston North, New Zealand; [3]Raumati Veterinary Centre, Raumati Beach, New Zealand.
[*]Corresponding author. Email: J.Munday@massey.ac.nz

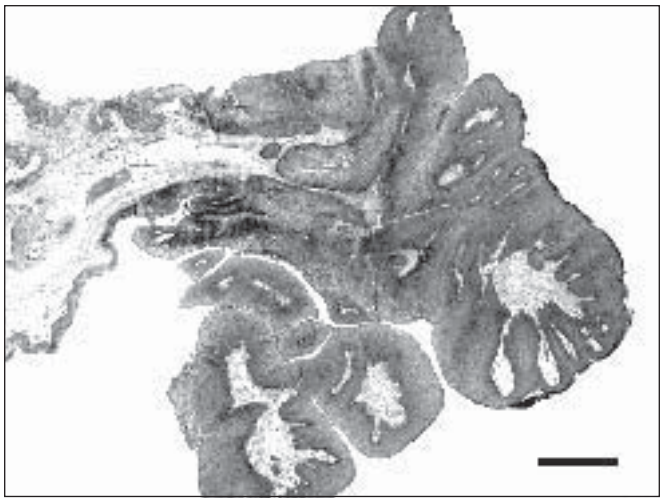

Figure 1. Conjunctival papilloma from a rabbit. The papilloma consisted of focal marked epidermal hyperplasia. The hyperplastic epidermis was arranged in folds and was supported by a stalk of dermal connective tissue. Hematoxylin and eosin stain; bar, 0.25 mm.

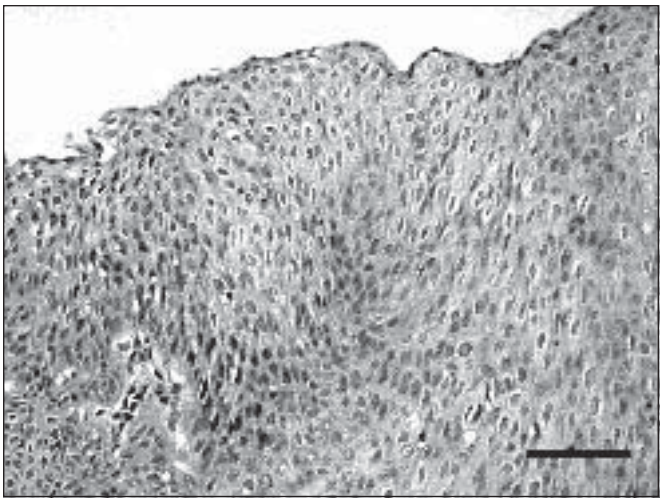

Figure 2. Conjunctival papilloma from a rabbit. Koilocytes containing a small dark nucleus surrounded by prominent perinuclear vacuolation were present within the superficial layers of the epidermis. Hematoxylin and eosin stain; bar, 33 µm.

under the cycling parameters of 94 °C for 10 min followed by 45 cycles of 94 °C for 1.5 min, 50 °C for 1.5 min, and 72 °C for 1.5 min with final extension at 72 °C for 5 min. Electrophoresis in a 1% agarose gel containing ethidium bromide was used to detect the 480-bp amplified fragment.

To sequence the DNA amplified from the papilloma, the PCR product was purified (Qiaex II 150 Gel Extraction Kit, Qiagen) and subjected to automatic dye-terminator cycle sequencing (BigDye Terminator Version 3.1 Ready Reaction Cycle Sequencing Kit, Applied Biosystems, Foster City, CA) by use of an automated sequencer (ABI3730 Genetic Analyzer, Applied Biosystems). Results were compared with known sequences from GenBank (http://www.ncbi.nlm.nih.gov/genbank) by use of the basic local alignment search tool (http://www.ncbi.nlm.nih.gov/blast). Sequencing of the PCR product from the rabbit conjunctival papilloma revealed 99.3% homology with ROPV DNA (GenBank accession no., AF227240). The occasional disparities between the sequence identified and that of the previously reported ROPV DNA were considered due to ambiguity within the chromogram rather than as evidence of a new subspecies of ROPV.

The mass from the nictitating membrane recurred 2 wk after surgical excision. At this time, the rabbit was noted to have lost weight. Weight loss continued for 3 mo until the rabbit died. The rabbit was not seen by a veterinarian during the period of weight loss, and a necropsy examination was not performed. The rabbit's owners reported that the papillomas were still present when the rabbit died.

Discussion

This case was unusual due to the location of the papillomas, the age of the rabbit, and the persistence of the papillomas. Although the virus is a mucosal-adapted papillomavirus,[5] natural ROPV-induced papillomas have only been reported to occur within the oral cavity. Viral transmission is thought to be from dam to kit during nursing,[2] possibly explaining the typical restriction of ROPV-induced papillomas to the oral cavity. Although natural ROPV-induced papillomas have only been reported to occur in the mouth, experimental inoculation of ROPV into genital tissue results in papilloma formation.[5] In the present report, the rabbit developed viral papillomas on a previously unreported mucous membrane, the conjunctiva. Whether the papillomavirus infection of the conjunctiva occurred during nursing or was due to later contact with another rabbit is unknown.

Rabbits are thought to become infected with ROPV at an early age.[2] Once in the oral cavity, the virus remains latent until damage to the oral mucosa allows papilloma development.[2] Because of the early age at infection, viral papillomas typically are seen in rabbits younger than 18 mo.[11] The rabbit in the present report was 3 y old when the conjunctival papilloma was observed. Although infection may have occurred during suckling, with the virus remaining latent in the conjunctiva for 3 y, the older age of the rabbit may support direct viral transmission from another rabbit at a later age. The presently reported rabbit had contact with other rabbits for 2 y prior to papilloma development.

The repeated recurrence of the papilloma after surgical excision was unusual. Most rabbit oral papillomas regress within 60 d.[6] The papillomas in the present case persisted for a total of 7 mo until the death of the rabbit. Regressing ROPV-induced papillomas contain prominent infiltrates of predominantly CD4+ lymphocytes.[13] None of the 3 papillomas examined histologically showed any evidence of an inflammatory infiltrate, suggesting that regression was not occurring. Perhaps effective immune response is more difficult to achieve within the conjunctiva than the oral cavity. Increased numbers of cells expressing CD11c were reported to be the earliest change in regressing papillomas.[13] These cells were considered likely to be dendritic cells,[13] and more dendritic cells may be present in the mouth than the conjunctiva. Alternatively, the rabbit showed progressive weight loss, culminating in death 3 mo after papilloma excision. Immunosuppressive conditions in humans allow increased papillomaviral infection and persistence.[1,8,10] An underlying disease may have both killed the rabbit as well as impaired the immune system during the 3 mo before death. Immunosuppression could explain the persistence of the papillomas in this rabbit. It appears unlikely that ROPV infection caused the death of this rabbit.

To our knowledge, rabbit oral papillomatosis has only been reported in the United States, Mexico, and Europe.[2,7,11] The rabbit in the present report was born and bred in New Zealand. Currently only rabbits from commercial laboratory animal breeders can be imported into New Zealand. The described rabbit had access to only 1 pet rabbit and was never in contact

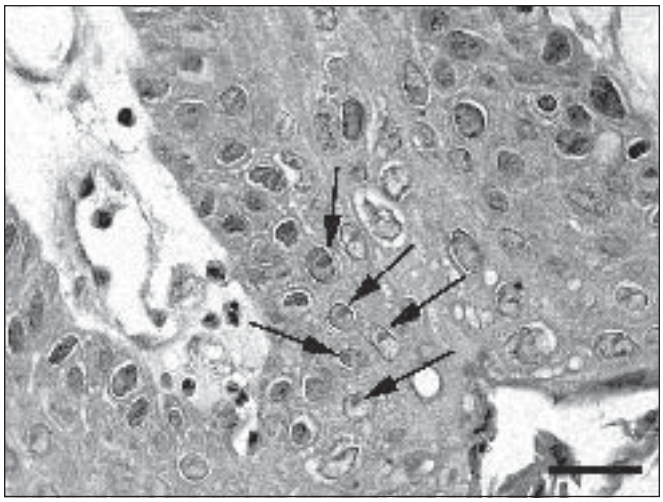

Figure 3. Conjunctival papilloma from a rabbit. Small numbers of keratinocytes contained small, round, deeply eosinophilic intranuclear bodies (arrows). These were considered consistent with papillomaviral inclusions. Hematoxylin and eosin, bar, 12 µm.

with any recently imported or laboratory rabbits. Worldwide, rabbit oral papillomatosis has been reported infrequently, but surveys of clinically normal young rabbits noted oral papillomas in 5%,[2] 17%,[12] and 31%[11] of animals. Because rabbits can be infected asymptomatically, the disease likely was imported into New Zealand in clinically normal rabbits.

Two closely related papillomaviruses infect rabbits.[9] ROPV usually causes oral papillomas in young rabbits. In contrast, CRPV results in cutaneous papillomas in rabbits of any age.[9] In the present case, the eyelid involvement and the older age of the rabbit was considered more consistent with CRPV than ROPV. Definitively differentiating between these papillomaviruses was important in this case for 2 reasons. First, papillomas due to CRPV in domestic rabbits often progress to squamous cell carcinoma.[9] As ROPV-induced papillomas never undergo malignant transformation,[6] definitive identification of the causative virus was prognostically important. Second, cottontail rabbits (*Sylvalagus* spp.) are the natural host of CRPV, with transmission of the virus to domestic rabbits by biting insects.[9] Cottontail rabbits are not present in New Zealand, and the identification of CRPV would have suggested that the virus had adapted to a new natural host in New Zealand.

Polymerase chain reaction was used to make a definitive diagnosis of ROPV in this case. This technique is valuable for identifying organisms in formalin-fixed paraffin-embedded tissue. The primers used have been shown to amplify DNA from a wide range of human and animal papillomaviruses.[3] The use of consensus primers meant that sequencing was required to identify the specific causative virus. Immunohistochemistry can be used to detect the presence of papillomaviral antigen in histologic sections. Due to the characteristic histologic appearance of the lesions and the subsequent amplification of ROPV, immunohistochemistry was considered unnecessary in the described case.

In conclusion, this report is the first description of natural ROPV infection resulting in papilloma formation in a location other than the oral cavity. In addition, this rabbit was older than most rabbits that develop this disease. The conjunctival papillomas persisted longer than typical, suggesting that either the location of the papilloma or an underlying immunodeficiency in this rabbit delayed regression of the lesion. In addition, this rabbit represents the first reported case of ROPV in the southern hemisphere. Further, this case demonstrates the value of PCR and subsequent sequencing in making an etiologic diagnosis.

References

1. **Ahdieh L, RS Klein, R Burk, S Cu-Uvin, P Schuman, A Duerr, M Safaeian, J Astemborski, R Daniel, K Shah.** 2001. Prevalence, incidence, and type-specific persistence of human papillomavirus in human immunodeficiency virus (HIV)-positive and HIV-negative women. J Infect Dis **184:**682–690.
2. **Dominguez JA, EL Corella, A Auro.** 1981. Oral papillomatosis in two laboratory rabbits in Mexico. Lab Anim Sci **31:**71–73.
3. **Forslund O, A Antonsson, P Nordin, B Stenquist, BG Hansson.** 1999. A broad range of human papillomavirus types detected with a general PCR method suitable for analysis of cutaneous tumours and normal skin. J Gen Virol **80(Pt 9):**2437–2443.
4. **Giri I, O Danos, M Yaniv.** 1985. Genomic structure of the cottontail rabbit (Shope) papillomavirus. Proc Natl Acad Sci U S A **82:**1580–1584.
5. **Harvey SB, NM Cladel, LR Budgeon, PA Welsh, JW Griffith, CM Lang, ND Christensen.** 1998. Rabbit genital tissue is susceptible to infection by rabbit oral papillomavirus: an animal model for a genital tissue-targeting papillomavirus. J Virol **72:**5239–5244.
6. **Hu J, NM Cladel, LR Budgeon, ND Christensen.** 2004. Characterization of three rabbit oral papillomavirus oncogenes. Virology **325:**48–55.
7. **Mews AR, JS Ritchie, CH Romero-Mercado, GR Scott.** 1972. Detection of oral papillomatosis in a British rabbit colony. Lab Anim **6:**141–145.
8. **Orth G.** 2006. Genetics of epidermodysplasia verruciformis: insights into host defense against papillomaviruses. Semin Immunol **18:**362–374.
9. **Percy DH, SW Barthold.** 2001. Pathology of laboratory rodents and rabbits. Ames (IA): Iowa State University Press.
10. **Rose B, D Wilkins, W Li, N Tran, C Thompson, Y Cossart, K McGeechan, C O'Brien, J Eris.** 2006. Human papillomavirus in the oral cavity of patients with and without renal transplantation. Transplantation **82:**570–573.
11. **Sundberg JP, RE Junge, MO el Shazly.** 1985. Oral papillomatosis in New Zealand white rabbits. Am J Vet Res **46:**664–668.
12. **Weisbroth SH, S Scher.** 1970. Spontaneous oral papillomatosis in rabbits. J Am Vet Med Assoc **157:**1940–1944.
13. **Wilgenburg BJ, LR Budgeon, CM Lang, JW Griffith, ND Christensen.** 2005. Characterization of immune responses during regression of rabbit oral papillomavirus infections. Comp Med **55:**431–439.