

Case report

Lymphoma with cutaneous involvement in three domestic rabbits (*Oryctolagus cuniculus*)

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(Received 28 September 1998; accepted 18 December 1998)

Abstract Three domestic rabbits (*Oryctolagus cuniculus*) with cutaneous lymphoma are described. Two rabbits were young (7 weeks and 1 years) and were euthanized within 1 week of showing clinical signs. Lymphoma was found in the skin and internal organs. The third rabbit was 9.5 years of age, and lived for more than 1 year after diagnosis. No response was seen to either 2 months of alpha-interferon administration or a 2.5-week course of isotretinoin treatment. After 1 year the rabbit died suddenly; the owner refused necropsy. Immunologic stains of the tumour in all three rabbits showed T cells to be the lymphoma cell type.

Keywords: alpha-interferon, cutaneous lymphoma, lymphoma, rabbit, retinoids, skin.

INTRODUCTION

Lymphosarcoma has been extensively reported in the rabbit.^{1–12} It has generally been acknowledged as a disease of young rabbits between 5 and 13 months of age.^{2–5,13} Wire-hair rabbits have a genetic predisposition to lymphosarcoma,⁴ with evidence pointing to a viral agent.^{6,7} In addition, viral particles have been noted on electron microscopy of lymphosarcoma of a New Zealand White rabbit.³ In three cases, rabbits developed lymphoma after being injected with viruses: a reovirus isolated from a human case of Burkitt's lymphoma,⁸ feline leukaemia,⁹ and an Epstein–Barr-virus-related herpesvirus.¹⁰ Most cases of lymphosarcoma in rabbits are aleukaemic, but cases of lymphoblastic or myeloid leukaemia in association with lymphosarcoma have been reported.^{5,11–13} In two reports the malignant lymphocytes were shown to be of T-cell origin.^{5,10}

Cutaneous lymphoma in domestic animals has been reported most commonly in dogs,^{14–16} cats,^{15,17}

horses,^{18–20} and hamsters.²¹ In the one reported case of cutaneous lymphoma in the rabbit multiple painless subcutaneous swellings were noted in an 18-month-old female Netherlands dwarf rabbit.²² Upon obtaining the diagnosis of cutaneous lymphoma by biopsy, the rabbit was euthanized and a necropsy performed. No gross or microscopic evidence of lymphosarcoma was seen in any other organ system. No viral particles were seen on electron microscopic examination of the neoplasm. One other rabbit with lymphoma has been reported which had a lesion in the auditory meatus.¹³

We report herein three rabbits with lymphoma involving the skin.

CASE REPORTS

Case 1

An approximately 1-year-old female New Zealand White laboratory rabbit weighing 5.4 kg was presented to Department of Pathobiology, The School of Veterinary Medicine, University of Pennsylvania for anorexia and lethargy of less than 1-week duration. The rabbit previously had been immunized with a cell line containing human epidermal growth factor (EGF) receptors; the last injection was given 8 months prior

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to the onset of the anorexia and lethargy. Physical examination showed a bilateral serosanguineous nasal discharge and an area of erythematous alopecia with haemorrhagic crusts extending from the lips and chin to the ventral neck (Fig. 1). Other areas of alopecia and erythema involved the axilla, caudal ventrum, and proximal hindlimbs. Within some of these areas were multiple firm erythematous cutaneous plaques. Generalized peripheral lymphadenopathy was present. Differential diagnoses included bacterial or viral dermatitis, dermatophytosis, ectoparasites, and neoplasia. Because infection with *Treponema cuniculi* (rabbit syphilis) was among the differential diagnoses, the rabbit was euthanized because of concerns of contagion. Immediately prior to euthanasia, blood was taken for a complete blood count (CBC) which showed a mild anaemia ($4.86 \times 10^6 \mu\text{L}^{-1}$, normal 5.5–7.5) and a mild relative lymphopenia ($1.55 \times 10^3 \mu\text{L}^{-1}$ [21%, normal 30–85%]).²³

Gross necropsy findings showed a rabbit in good body condition, with numerous pale tan nodules in the lungs and depressed bilateral areas in the renal cortices. Histological examination with routine haematoxylin & eosin (H&E) staining showed infiltrates of neoplastic lymphocytes in the skin, lymph nodes, and lungs. The lymphocytes in the skin frequently occupied the entire dermis, and infiltrated the epidermis (Fig. 2). In other areas, the lymphocytes had a perifollicular/periadnexal (Fig. 3). The kidneys showed only mild tubular interstitial nephritis.

In order to cell-type the cutaneous lymphocytic infiltrate, skin sections were stained with a standard streptavidin–biotin method previously described,²⁴ with the following modifications for paraffin sections. Four- μm sections were deparaffinized in xylene and hydrated through graded alcohols to phosphate buffered saline buffer. Just prior to the application of the primary antibody, sections were pretreated with microwave-induced boiling in citrate buffer pH 6 for 20 min and allowed to set at room temperature (RT) for 30 min. The primary antibody rat anti-CD3 monoclonal (Novocastra Laboratories Ltd, Vector Labs, Burlingame, CA, USA) was used at a 1:5 dilution and incubated at RT for 1 h 15 min. Positive and negative controls were performed. Staining of the skin sections for CD3 showed large numbers of positive cells, confirming the T-cell origin of the neoplasia (Fig. 4).

Case 2

A domestic 7-week-old female Netherland dwarf rabbit weighing 0.9 kg was presented to the Exotic and Laboratory Animal Services, Royal (Dick) School of Veterinary Studies, University of Edinburgh for a 2-day history of bilateral blepharitis (Fig. 5). Physical examination revealed an alert rabbit with no other abnormalities. The most likely differential diagnoses considered at the time were viral (myxomatosis) or bacterial (*T. cuniculi*) infection. Microbiologic culture of the conjunctival swabs was negative. The rabbit was treated with chlortetracycline ophthalmologic

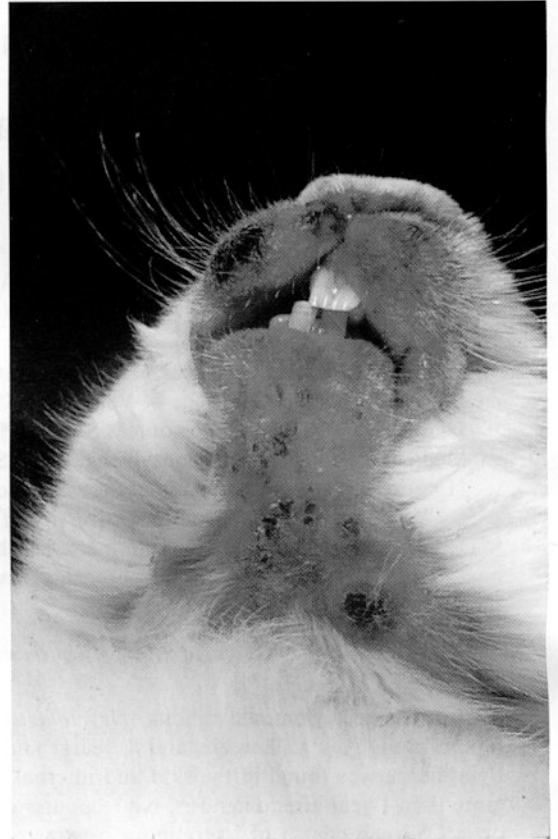
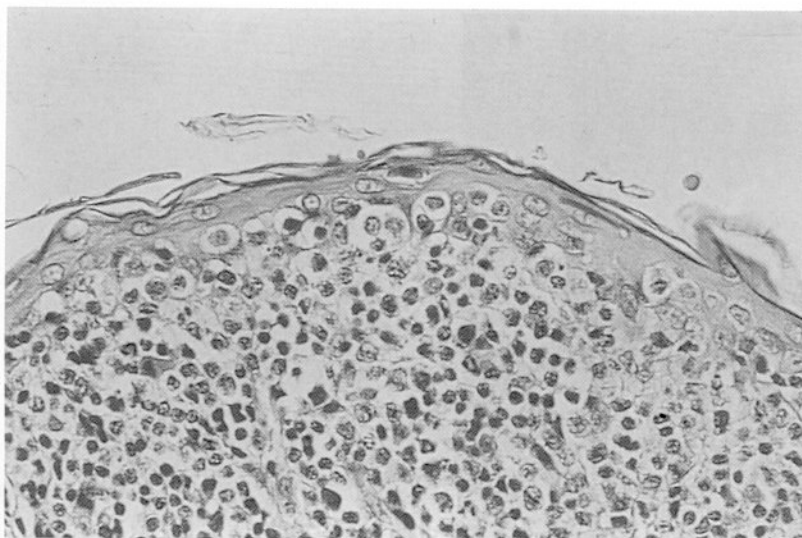


Figure 1. Case 1, 1-year-old female New Zealand White rabbit with cutaneous lymphoma. Notice erythematous alopecia with haemorrhagic crusts extending from the lips and chin to the ventral neck.

drops for 3 days with no response and subsequently a neomycin/dexamethasone ophthalmologic ointment for 2 days. During this time the rabbit became dyspneic, and the owners requested euthanasia. Gross necropsy showed a rabbit in good body condition, with bilaterally swollen palpebrae. Superficial and deep (especially submandibular, retropharyngeal, and mesenteric) lymph nodes were markedly enlarged and firm, with cream–red discoloration. Both lungs had reddened areas in some lobes. The liver was hyperpigmented (darker in colour than expected).

On histological examination with routine H&E staining, the lungs had a marked perivascular, peribronchiolar and subpleural infiltrate of large pleomorphic round cells. These had round, oval, and cleaved nuclei and mostly indistinct cytoplasm. Mitotic figures were common, and areas of necrosis and apoptosis were noted. Similar infiltrates were found in the skin, liver, kidney and heart, in a primarily perivascular arrangement. Sheets of these cells obliterated the normal architecture of the lymph nodes. The infiltrate in the skin was primarily in the subcutis and deep dermis (Fig. 6). Immunologic staining was performed as described for case 1. Staining of the skin sections for CD3 showed large numbers of positive cells, confirming the T-cell origin of the neoplasia, although the staining of the cells was somewhat weaker than for case 1.



cutaneous lymphoma

Figure 2. Photomicrograph, case 1. Notice tumour cells infiltrating the epidermis, leaving only the stratum corneum intact (H&E $\times 400$).

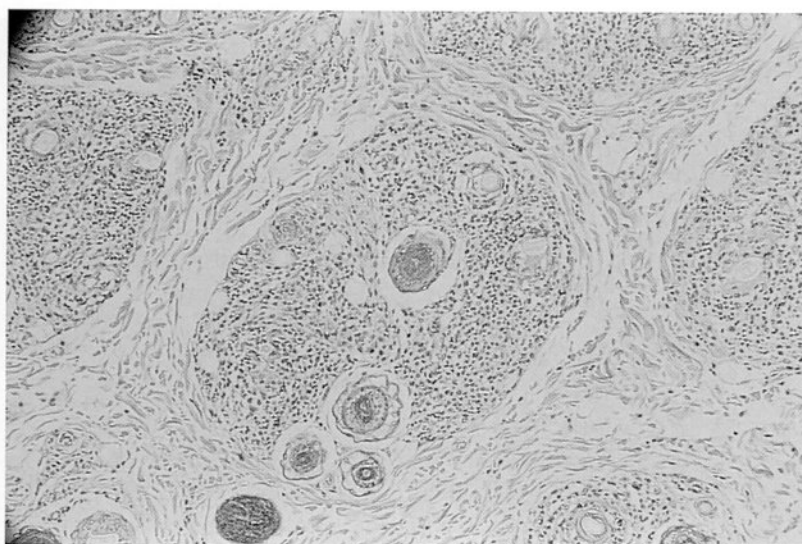


Figure 3. Photomicrograph, case 1. Notice tumour cells infiltrating around hair follicles and adnexa (H&E $\times 100$).

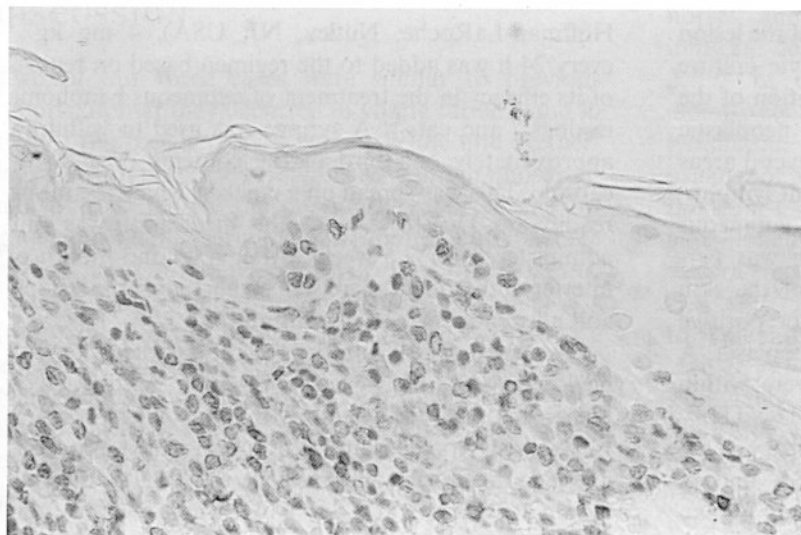


Figure 4. Photomicrograph, case 1. CD3 stain: tumour cells stain brown in contrast to haematoxylin counterstain. Tumor cells are seen in the dermis and are infiltrating the basal cell layer of the epidermis ($\times 400$).

Case 3

A 9.5-year-old male domestic rabbit weighing 2.5 kg was presented to the Colorado State University-Veterinary Teaching Hospital (CSU-VTH) with a 1-

week history of nonpruritic alopecia. The alopecia was confined to an area on the left lateral thorax. The most probable differential diagnoses considered at the time were dermatophytosis or trauma. Erythema and

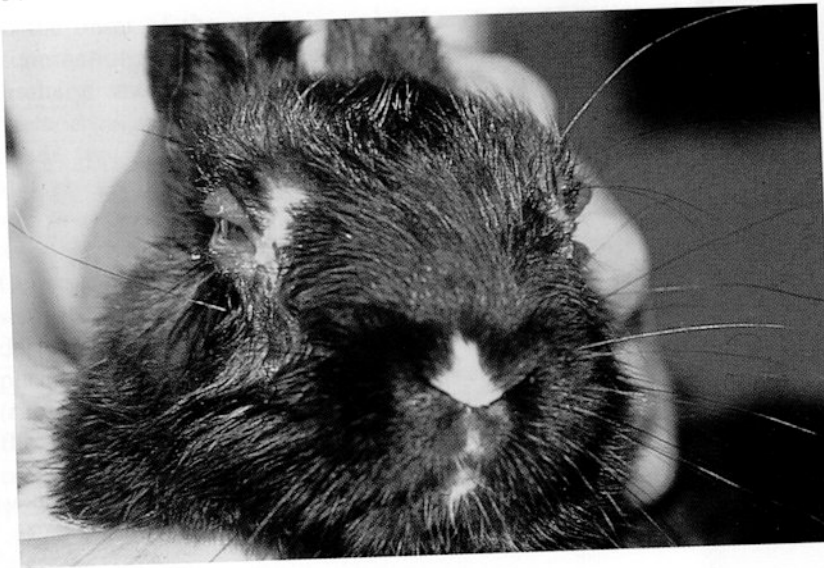


Figure 5. Case 2, 7-week-old female Netherland dwarf rabbit with cutaneous lymphoma. Notice the bilateral blepharitis.

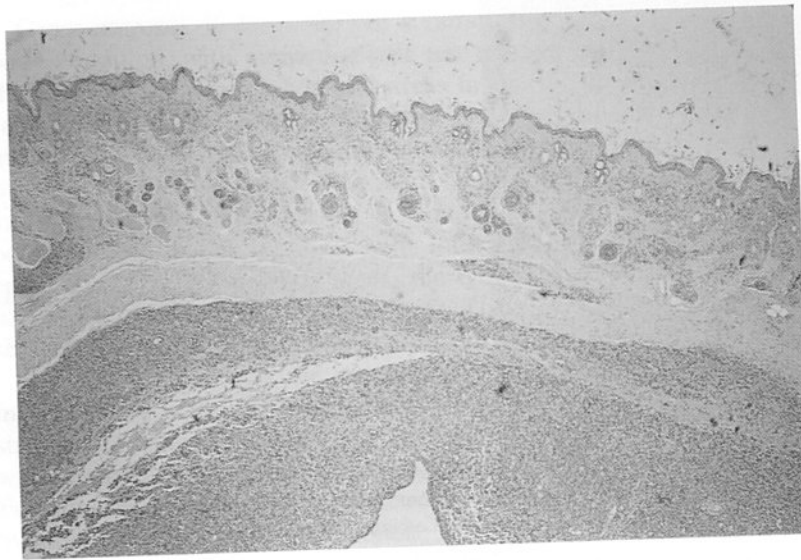


Figure 6. Photomicrograph, case 2. Notice the infiltrate in the skin, primarily in the deep dermis (H&E $\times 40$).

bruising were noted. An impression smear of the lesion showed many lymphocytes. A dermatophyte culture was negative. A skin biopsy showed infiltration of the superficial dermis and the epidermis with neoplastic lymphocytes with routine H&E staining. Several areas of the epidermis had collections of malignant lymphocytes (Pautrier's abscesses) consistent with cutaneous lymphoma (Fig. 7). Immunologic staining was performed as described for case 1. Staining of the skin sections for CD3 showed large numbers of positive cells, confirming the T-cell origin of the neoplasia. A CBC and a serum biochemistry profile were within normal laboratory values for rabbits at CSU-VTH.

Based on reports of alpha interferon's efficacy in the treatment of T-cell lymphoma in humans²⁵ and in dogs,²⁶ the rabbit was treated with recombinant human interferon alpha-2b (Intron A; Schering, Kenilworth, NJ, USA), at a dosage of 1.5 million units per m². This was given subcutaneously three times weekly for 1 month. At the end of this time the skin of the lesion was more erythematous and thickened. Interferon treatment was continued, and isotretinoin (Accutane;

Hoffman-LaRoche, Nutley, NJ, USA), 4 mg kg⁻¹ every 24 h was added to the regimen based on reports of its efficacy in the treatment of cutaneous lymphoma in dogs¹⁶ and cats.¹⁷ A syringe was used to withdraw approximately one-third of the contents of a 20-mg capsule. This was spread on a cracker, which the rabbit readily ate. Due to expense, this medication was only administered for 2.5 weeks. After a second month of interferon treatment there was no change in the lesions, and all treatment was discontinued.

Over the next 10 months the rabbit was otherwise healthy except for a 2-week episode of sneezing which responded to treatment with antibiotics. Serum biochemistry profiles and CBCs performed 6 months and 8 months after diagnosis were within the normal ranges for rabbits at CSU-VTH. Eight months after the diagnosis of cutaneous lymphoma, additional areas on the body developed alopecia, erythema, and scaling, especially on the head and neck (Fig. 8). One year after the clinical signs were first noted, the rabbit had a sudden 24 h episode of weakness which progressed to death. The owner refused necropsy.

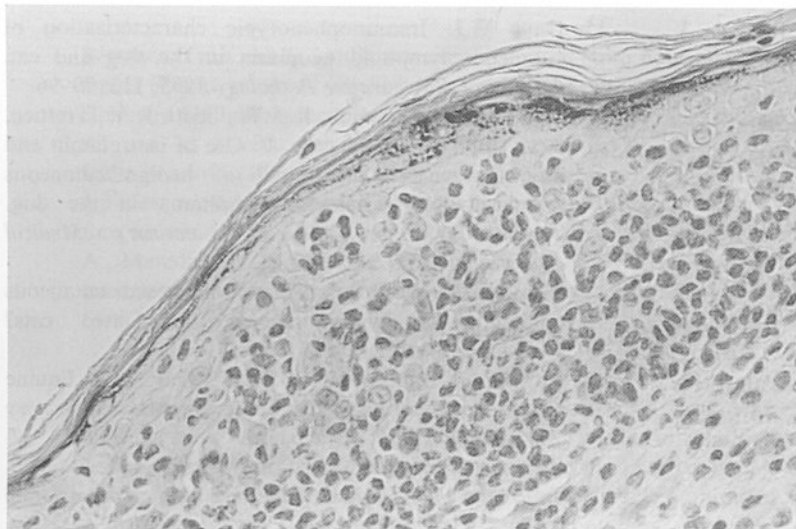


Figure 7. Photomicrograph, case 3. Notice the infiltration of malignant lymphocytes in the epidermis forming Pautrier's abscesses (H&E $\times 400$).



Figure 8. Case 3. Notice alopecia, erythema and scaling of the head and neck.

DISCUSSION

Two of our three cases were similar to previous reports, in that they occurred in young rabbits, and in two of the breeds (New Zealand White and Netherlands Dwarf) reported previously with lymphosarcoma.^{1-3,5,13,22} Unlike the previously reported case of cutaneous lymphosarcoma in a rabbit,²² both of these rabbits had lymphoma cells in various other organs. While the rate of progression of the disease in case 1 (the New Zealand White) is unknown, the disease appeared to progress quite rapidly in case 2 (the Netherlands Dwarf) as the rabbit deteriorated over the course of 1 week. Case 3 was different in that it occurred in an older rabbit, and had a relatively slow progression of clinical signs. Histological findings in this rabbit, and the New Zealand White, showed epitheliotropism, suggestive of T-cell lymphoma (mycosis fungoides [MF]) as seen in other species.^{14-17,19,21} Cytologic staining for CD3 confirmed that the invading lymphocytes were T cell in origin as is found in MF. However, nonepitheliotropic T-cell lymphoma has been reported in the dog,¹⁵ cat,¹⁵

horse¹⁸ and humans,²⁷ and may have the malignant infiltrate deep in the dermis or subcutis, as did case 2.^{27,28} In cases 1 and 2 we are unable to say definitively whether these two rabbits had cutaneous lymphoma which then metastasized to the internal organs, or visceral lymphoma which metastasized to the skin. Cutaneous lymphoma, both epitheliotropic and nonepitheliotropic, which metastasize to the viscera has been well documented in other species.^{17,29-32} The relatively long-term survival (relative to typical life expectancy) of case 3 is similar to that seen in other species with mycosis fungoides.^{18,21,29} This fact, coupled with the epitheliotropic T-cell infiltrate, was most supportive of a diagnosis of mycosis fungoides.

None of the rabbits in this report had been injected with viral agents, and cases 1 and 3 had aleukaemic CBCs. In case 3 the touch preparation for cytology showing many lymphocytes was probably due to the presence of these cells in the skin.

Case 1 was a laboratory rabbit and the potential for contagion due to the suspect *Treponema cuniculi* outweighed concern for treatment. Case 2 deteriorated

rated before a diagnosis could be made. In case 3, based on reports in other species, isotretinoin and alpha-interferon-2b were administered. While there was no response to either drug, the period of time that the rabbit was treated with isotretinoin may have been too short to expect clinical improvement, although some dogs may show substantial improvement to retinoids within 2 weeks.¹⁶

The lesions in these rabbits were similar to other more-common skin diseases in this species. Clinicians should not hesitate to biopsy scaling, crusting, erythematous, or blepharitic lesions when presumptive treatment for more common maladies is ineffective.

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