Fatal herpesvirus infection in commercial rabbits

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Abstract

Acute mortality occurred in two unrelated rabbitries. In the rabbits examined, an unidentified herpesvirus caused lesions that have not been reported previously in this species. The primary lesions were multifocal hemorrhagic dermatitis on the face and back, localized pneumonia, and severe splenic necrosis. Large eosinophilic, intranuclear inclusion bodies that were observed in tissue sections of skin, spleen, and lung were identified as herpes-like viral particles by electron microscopy, and herpesvirus was cultured on rabbit kidney cells. Intramuscular injection of tissue culture fluid containing virus resulted in mortality and severe illness in two seven-week-old domestic rabbits four and six days postinfection, respectively. The gross and microscopic lesions were reproduced and herpes-like viral inclusions were observed in skin lesions. Herpesvirus was recovered from lung, trachea, spleen, liver, and from the thigh muscle at the site of inoculation. The experimental infection also activated severe pasteurella septicemia. The herpesvirus isolate needs further characterization.

Résumé

Infection fatale par le virus herpès chez le lapin destiné à la consommation

Des mortalités subites sont survenues dans deux élevages non apparentés de lapins. Chez les animaux examinés, un virus herpès non identifié a causé des lésions qui n'ont pas été décrites auparavant chez cette espèce. Les principales lésions ont été une dermatite hémorragique multifocale à la face et sur le dos, une pneumonie localisée et une nécrose sévère de la rate. De gros corps d'inclusion intranucléaires éosinophiliques, observés dans des sections de la peau, de la rate et des poumons, ont été identifiés par microscopie électronique comme étant des particules virales s'apparentant à un herpès. De plus, un virus herpès a été cultivé sur des cellules rénales de lapins. Une injection intramusculaire d'un bouillon de culture tissulaire renfermant des particules virales a causé une maladie sévère et la mort de deux lapins domestiques âgés de sept semaines, quatre et six jours après l'inoculation. Les lésions macroscopiques et microscopiques ont été reproduites et des corps d'inclusion "herpes-like" ont été observés dans les lésions cutanées.

Le virus herpès a été isolé à partir des poumons, de la trachée, de la rate, du foie et du site d'injection du muscle de la cuisse. L'infection expérimentale a aussi activé une septicémie sévère due à *Pasteurella*. L'isolat du virus herpès nécessiterait d'être davantage caractérisé. ***(Traduit par Dr Thérèse Lanthier)***

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Introduction

A herpes-like virus (named virus III) was first isolated from clinically normal domestic rabbits (*Oryctolagus cuniculus*) by Rivers and Tillett in 1924 (1). This virus was reisolated by Neshburn (2) during routine screening of primary kidney cell cultures prepared from healthy domestic rabbits (New Zealand albinos). The virus was named *Herpesvirus cuniculi* and has since been found at various infection rates in domestic rabbits of commercial and laboratory colonies (3,4). There are no reports of naturally occurring clinical disease associated with herpesvirus in rabbits of the genus *Oryctolagus*. We report herein fatal infections with an unidentified herpesvirus in two unrelated commercial rabbitries, and the experimental reproduction of the disease using the field strain isolate.

Materials and methods

Case 1

In June 1990, tissues from a pregnant New Zealand White doe (*O. cuniculus*) were submitted from a rabbitry in northeastern Alberta. This animal was one of two other periparturient does and 17 neonatal rabbits that died during one week. A breeding buck had been introduced in April of 1990 with purulent rhinitis typical of pasteurellosis. Most rabbits were found dead without clinical signs having been observed. A few were depressed and anorectic about eight hours before death. Fresh, frozen, and formalized tissues were available for histological and virological studies. Four remaining rabbits were acquired at two months of age for further studies. They showed no clinical signs. Two of the rabbits were injected intramuscularly (IM) once daily with 1 mg dexamethazone (Dexone-5, Sterivet Laboratories Ltd., Mississauga, Ontario) for five consecutive days to reactivate any latent infection. The two other animals served as controls. After 17 days, the rabbits were euthanized using ketamine IM (Ketaset, Ayerst Laboratories, Montreal, Quebec) as an anesthetic followed by intracardiac injection of an overdose of pentobarbital (Euthanyl, MTC Pharmaceuticals, Cambridge, Ontario) for postmortem examination.

Case 2

Three dead adult Rex rabbits (*O. cuniculus*) were submitted from a rabbitry in northern British Columbia in August 1990. This operation houses about 200 animals. Four weeks earlier, a buck was purchased from a neighbor who raises rabbits and attended a show in Alberta during that summer. The owner noticed affected rabbits with swollen eyes and faces. A few animals had nasal discharge. Pasteurellosis was endemic in the herd, and affected animals had been treated with an unspecified antibiotic by the owner. Eight older breeding stock animals and two young of the year had died and two more were reported sick. Rabbits used for pelting were housed in a shed sepa-

rate from the breeding group and were not affected. Routine necropsy procedures were followed and lesions were submitted for histological, bacteriological, and virological examinations.

Virological cultures

A tissue homogenate for viral isolation was prepared from the pool of spleen, skin lesions, lung, liver, kidney, intestine, and conjunctival tissue. These tissues were ground with a mortar and pestle and suspended at a 20% concentration in minimum essential medium (MEM, Gibco Laboratory, Burlington, Ontario) containing 10% fetal bovine serum (Sigma Chemical Co., St. Louis, Missouri, USA). Viral isolation was attempted in rabbit kidney cell tube cultures (RK13 cell line, American Type Culture Collection, Rockville, Maryland, USA). One mL of kidney cell suspension with a concentration of 100,000 cells per mL was seeded in a tube and fed with MEM supplemented with 10% fetal bovine serum for 4–6 h at 37°C. At 60% confluence, each cell culture was inoculated with 0.2 mL of the tissue homogenate. The medium of the cell culture was replaced with MEM supplemented with 2% fetal bovine serum 24 h postinoculation. Infected and control noninfected tube cultures of RK13 cells were examined daily under a phase contrast light microscope for cytopathic effect. Two consecutive passages were carried out to boost viral titer.

Cell-associated viral particles were released by freezing and thawing the infected cells three times in phosphate buffered saline at pH 7.2. Negatively stained grids were prepared and examined using a Philips model 201 electron microscope (Philips Electronics Ltd., Scarborough, Ontario).

Virus inoculum

The inoculum consisted of MEM tissue culture fluid with 2% fetal bovine serum using 1 mL of 10^{-1} tissue culture infective doses.

Experimental reproduction of the disease

Five seven-week-old domestic meat-type rabbits were housed in pairs or singly in large wire floor cages and provided with free choice rabbit pellets (Masterfeeds, Edmonton, Alberta), hay, and water. They were on photoperiod cycles of 11 h light, 13 h dark. Housing and all procedures followed the guidelines of the Canadian Council on Animal Care. After an observation period of 13 days, a blood sample was obtained from each animal and the following injection schedule was administered:

- Rabbit 1: housed with rabbit 2, 1 mL tissue culture fluid only, IM into the semitendinosus muscle.
- Rabbit 2: 1 mL viral suspension IM into the semitendinosus muscle.
- Rabbit 3: housed with rabbit 4, uninoculated control.
- Rabbit 4: 1 mL viral suspension IM into the semitendinosus muscle.
- Rabbit 5: housed singly but in the same battery cage system as the other animals, 1 mL tissue culture fluid only, IM into the semitendinosus muscle.

The animals were observed daily and rectal temperatures were recorded. Animals were euthanized using 40 mg/kg ketamine IM as an anesthetic followed by intracardiac inoculation of an overdose of pentobarbital. Blood samples were collected for complete white blood cell counts prior to euthanasia. Tissues were collected at necropsy for microscopic examination and for bacteriological and virological cultures.

Results

Case 1

The significant findings at necropsy of the doe reported by the submitting practitioner included pulmonary congestion and edema, hydrothorax, and ecchymoses in the spleen, kidneys, stomach, and intestines. There were moderate numbers of cutaneous hemorrhagic macules scattered over the body. Microscopically, throughout the splenic red pulp there was severe acute necrosis, fibrinous inflammation, and hemorrhage. A few syncytial cells were scattered throughout, containing large intranuclear eosinophilic or heterophilic inclusion bodies (Figure 1). Foci of acute necrosis and hemorrhage were in the dermis, lung, and adrenal gland with many intranuclear inclusions in degenerative cells in the dermis and lung. There was mild acute centrilobular hepatic degenerative and fatty change whereas kidney, heart, pancreas, stomach, intestine, and uterus had no significant lesions. Electron microscopic examination of inclusion bodies in paraffin sections revealed numerous herpesvirus-like particles in nuclei and cytoplasm. Rabbit kidney cell cultures inoculated with pooled lung, liver, spleen, kidney, and skin samples showed cytopathic changes by five days postinoculation. Electron microscopy revealed viral particles consistent with the morphology and size of a herpesvirus (Figure 2). No pathogenic bacteria were isolated from cultures of lung, kidney, and spleen. Gross and microscopic examination of the four rabbits submitted live failed to detect any evidence of viral infection.

Case 2

All three rabbits were in excellent bodily condition. One animal had pronounced edema over the rostrum. All had 1.0–1.5 cm circular, red, raised skin lesions on the face, under the chin, over the lumbar area and to a lesser extent on the sides and abdomen (Figure 3). One had a slight nasal discharge and edema of the palpebral conjunctiva. Submandibular lymph nodes were markedly enlarged. The spleens had large irregular pale areas, sometimes with dark red centers (Figure 4). On microscopic examination, the skin lesions were focal areas of ballooning degeneration of epithelium with formation of vesicles. These areas were infiltrated by mononuclear cells (Figure 5) with scant cytoplasm but large intranuclear inclusion bodies (Figure 6). In the dermis there were lymphocytic infiltrations, foci of necrosis and neutrophilic infiltration, and edema. The spleens had large areas of necrosis with organized fibrin and fibroblast infiltration. The submandibular lymph nodes were densely populated by lymphocytes mixed with some plasma cells. Subcapsular sinuses sometimes contained fibrin and the capsule was thickened by fibrin, and protein-

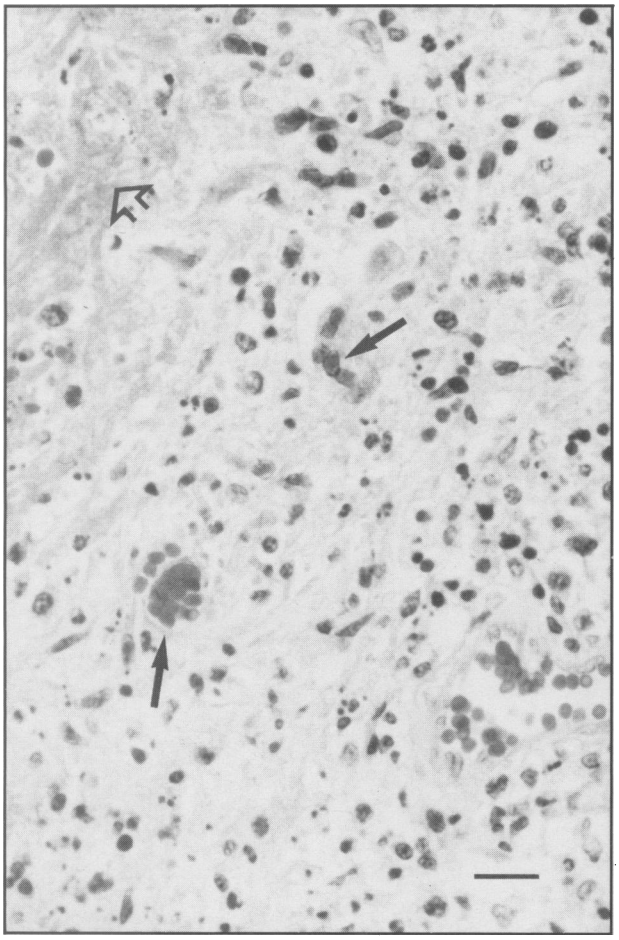

Figure 1. Spleen from a spontaneous case of herpesvirus infection in a New Zealand white doe (Case 1). Syncytial cells (dark arrows) contain intranuclear inclusions. There is widespread necrosis and fibrinous exudate (open arrow). H&E. Bar = 17 μm.

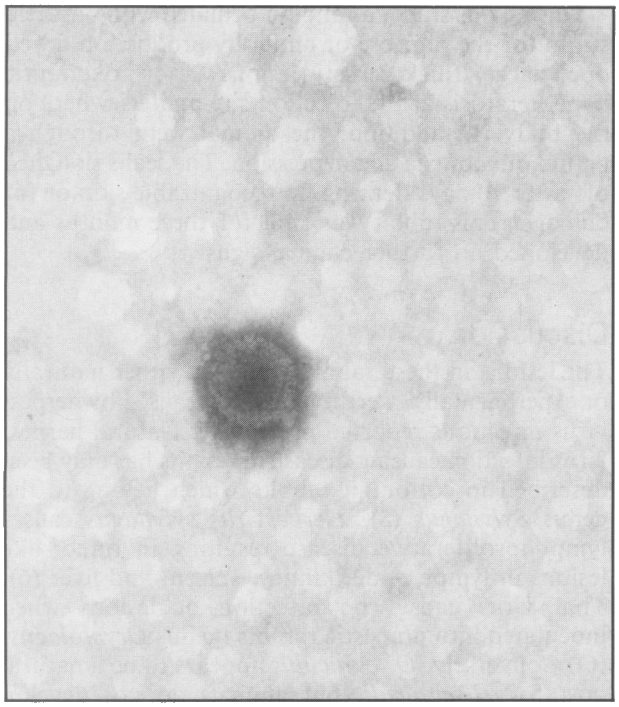

Figure 2. Herpesvirus isolated from pooled rabbit tissues from case 1 × 33500.

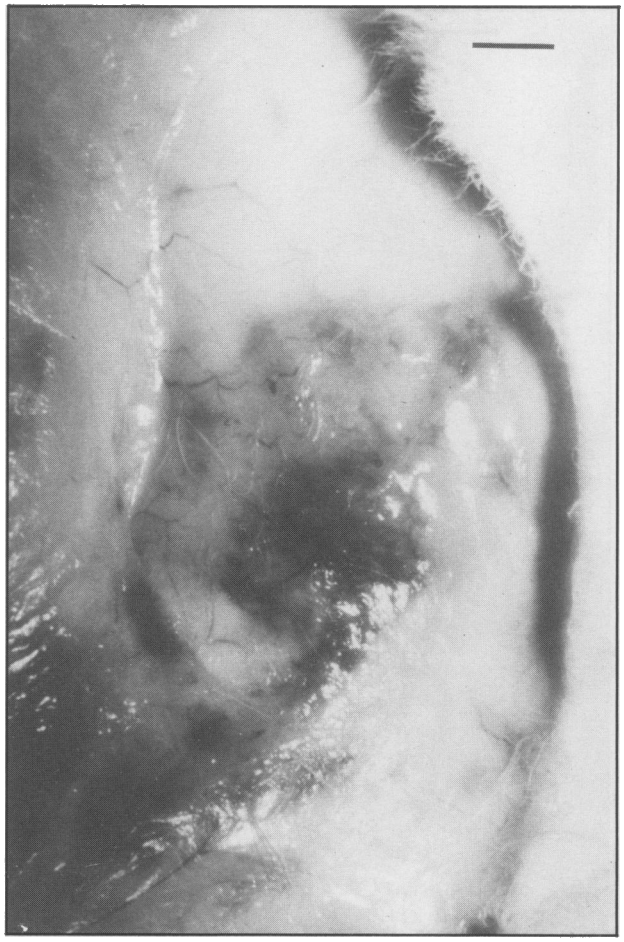

Figure 3. Subcutaneous view of a red, raised skin lesion from the back of a domestic rabbit with a naturally-occurring herpesvirus infection (Case 2). Bar = 0.3 cm.

aceous material with lymphocytic infiltration. Lungs were irregularly congested but in one animal there was a large area of alveolitis and fibrinous exudation. Livers and kidneys had no significant lesions. Bacterial cultures from eye, lymph nodes, liver, and spleen had no significant growth. A swab sample from the rabbit with the nasal discharge yielded *Neisseria* spp. No *Pasteurella* spp. was isolated. Electron microscopic examination of tissue recovered from paraffin sections showed numerous herpes-like viral particles. Tissue cultures from skin and conjunctiva inoculated onto RK13 cells yielded a herpesvirus.

Experimental disease

The virus-inoculated rabbit 2 had a slight fever of 41.2°C and was lethargic at three days postinoculation. It was found dead the next day. On day 6 postinoculation, rabbit 4 was very lethargic and had a milky ocular discharge. The rectal temperature had dropped from 40°C to 38.5°C. Rabbit 4 was killed together with the tissue culture-injected control animal 1. The two remaining rabbits showed no clinical signs and were killed at 34 days postinoculation. Comparison of preinoculation white blood cell counts with those taken six days postinoculation revealed an increase of 38% in total white blood cells to 14.9 × 10^9/L in the virus-inoculated rabbit. There was an absolute increase in neutrophils and monocytes

Figure 4. Extensive areas of necrosis (arrows) in spleen of rabbit from case 2.

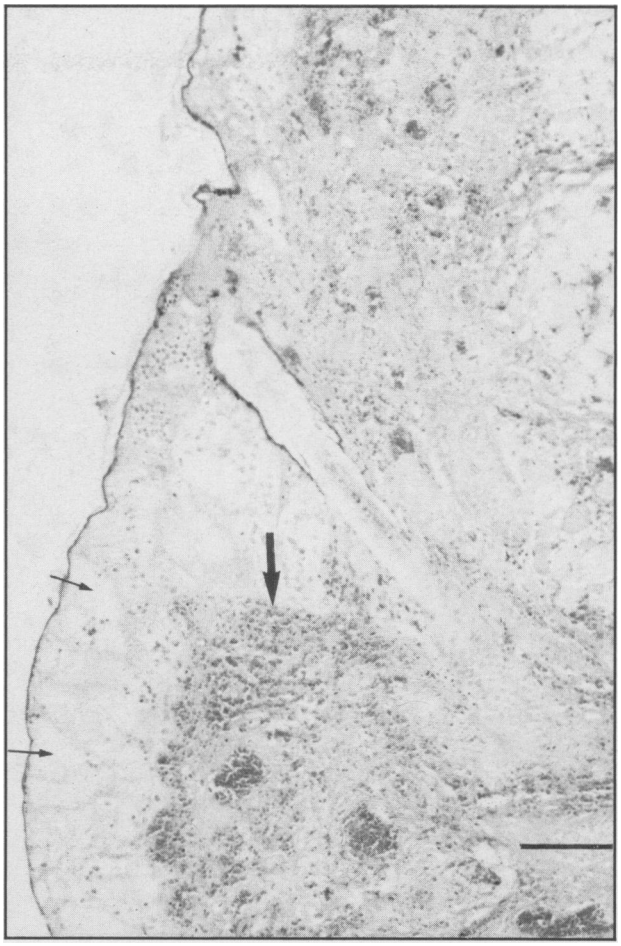

Figure 5. Epidermal ballooning degeneration (thin arrows) and dermal inflammation (thick arrow) in skin lesion from case 1 as shown in Figure 3. H&E. Bar = 100 μm.

but a drop in lymphocytes. Tissue culture and uninoculated controls remained at an average of 9.2×10^9/L.

Necropsy of the virus-inoculated animals revealed abundant straw-colored, gelatinous subcutaneous fluid over the injection site and in the inguinal area. The muscles were necrotic with dark brown discoloration. The noninjected thigh was normal. In contrast, the tissue culture-injected rabbits had no lesions at the injection site. The livers of the infected rabbits had pale, pinpoint foci throughout, and the spleens had large pale areas of necrosis, sometimes with a hemorrhagic periphery. The lungs were very congested, and the tracheas hyperemic. There was a crusty nasal discharge and a bilateral, turbid ocular discharge. Circular areas of erythema were found on the skin, primarily on the face and back. The entire thickness of the skin was involved. There was no alopecia.

Bacteriological cultures of both animals yielded a heavy growth of *Pasteurella multocida* from the lungs, necrotic thigh muscle, liver, spleen, and subcutaneous fluid. Nasal swabs from rabbit 3, which was housed with the virus-infected rabbit 4, grew only *Neisseria* spp., whereas in the singly-housed tissue culture-inoculated rabbit 5, both *Neisseria* spp. and *P. multocida* were isolated from the nasal mucosa. The tissue culture-inoculated and uninoculated controls had no visible lesions. Individual virological cultures of lung, trachea, spleen, liver, and necrotic thigh muscles from virus-inoculated animals 2 and 4 all yielded herpesvirus.

During this study, a rabbit inoculated with our field strain for the purpose of antibody production developed large, thick, crusty lesions of orthokeratotic hyperkeratosis at the injection site and elsewhere on the body. In addition, the animal went through a period of anorexia and depression. The scabs sloughed off after 10 days, leaving no recognizable skin or fur damage. This rabbit was kept for three months and developed no further clinical signs.

Discussion

The lesions in these rabbits, infected either naturally or experimentally, were unlike those caused by herpesvirus infections reported previously. Natural herpesvirus infection causing disease in rabbits has only been described in cottontail rabbits which belong to the genus *Sylvilagus* (5). *Herpesvirus sylvilagus* causes lymphoproliferative disease resulting in tumor-like lesions in lymph node, kidney, spleen, and liver (6). That virus causes no infection or lesions when inoculated into domestic rabbits (genus *Oryctolagus*) (6). Conversely, *O. cuniculus* appears to be a natural host for *H. cuniculi*, but animals do not develop lesions even when inoculated intraperitoneally with the virus (7). Corneal swelling and localized erythema may

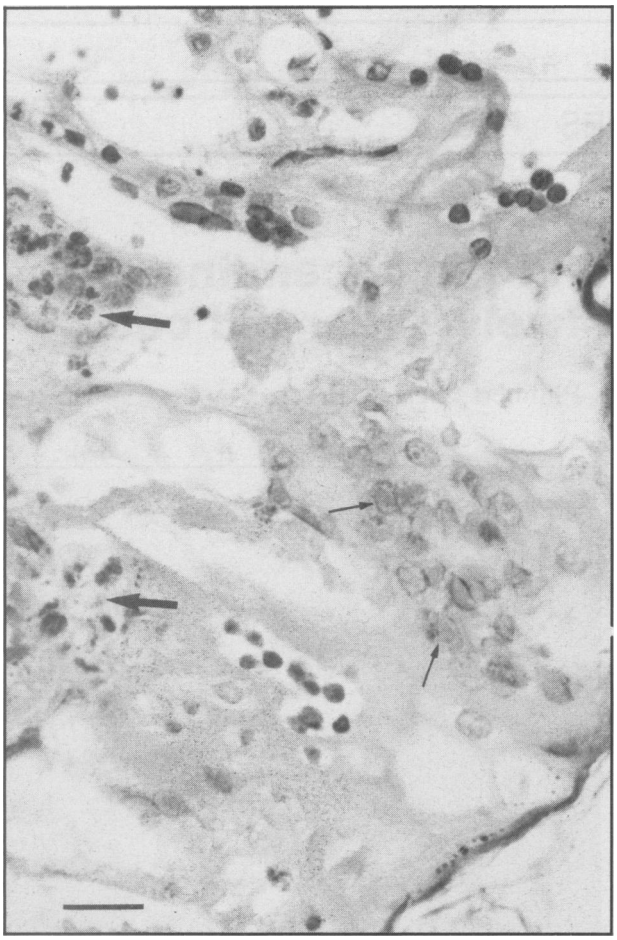

Figure 6. Higher magnification of Figure 5. Intranuclear viral inclusions indegenerative cells in the subepidermal layer (thin arrows) and inflammation and necrosis in dermis (thick arrows). H&E. Bar = 20.5 μm.

occur when inoculation is via corneal or dermal scarification (4).

The rabbit is used as a model for herpesviruses of other species such as the virus of bovine malignant catarrhal fever. In that case, intravenous or intraperitoneal infection results in lymphadenitis and splenic and thymic necrosis (8,9) but, unlike our cases, lesions are not observed in the skin or lung. The virus in our study appears to be more pathogenic. It apparently spreads quickly through all organ systems, including the skin, causing severe tissue changes, illness, and mortality. The changes in the leukocyte count may reflect a concurrent pasteurella septicemia rather than viremia, particularly with the increase in neutrophils. It may be argued that mortality in the experimentally-infected rabbits was due to pasteurellosis. However, in the field cases no concurrent pasteurella septicemia was apparent. It is possible that this herpesvirus might be involved in activating *P. multocida*, thereby leading to pasteurella outbreaks in rabbit colonies. The dexamethazone treatment did not activate any latent virus infection. This was also unsuccessful when corticosteroids were used in cottontail rabbits to induce viral shedding after experimental infection with *H. sylvilagus* (10). Recovery from the skin lesions of the rabbit used for antibody production reflects the histological evidence that the follicular epidermis was not involved. This allowed the haircoat to grow back but also points out the possibility that such lesions might be missed unless the animals are carefully examined. Furthermore, survival of this rabbit may represent the possibility of a carrier state for the virus.

The mode of transmission of herpesvirus among rabbits is not yet known. After failure of transplacental transmission studies (10) and insect vector studies (11), it was suggested that nasal or lacrymal excretions might be infective. In our study, contact animals remained healthy. The virus appears to be more virulent than *H. cuniculi*, but shares the ability to produce skin lesions similar to those described by Rivers and Tillett (1). The two field cases described in this study occurred far apart geographically; however, the possibility that the infection originated from a show which contact animals from both farms may have attended cannot be ruled out. The viral isolate remains to be characterized.

Acknowledgments

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