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Source: Journal of Wildlife Diseases, 17(3) : 471-477

Published By: Wildlife Disease Association

URL: <https://doi.org/10.7589/0090-3558-17.3.471>

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## PARMA WALLABY HERPESVIRUS INFECTION

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**Abstract:** Three Parma wallabies (*Macropus parma*) were inoculated with a herpesvirus recovered from a captive Parma wallaby with fatal naturally-occurring disease. Two intravenously inoculated animals died after 5 days and one animal infected via the conjunctiva and nasal mucosa was killed when moribund at 7 days. An additional two wallabies held in contact with the others became infected; they were killed at 11 days, when one was severely affected and one was mildly affected. All had small vesicles and ulcers of the skin of the upper and lower lips, eyelids, ano-genital area and adjacent genital mucosa. Small vesicles and ulcers and large ulcers, with adherent necrotic epithelium and inflammatory debris, were present on the mucosa of the upper lips and adjacent gums and the conjunctiva. Numerous large basophilic or eosinophilic intranuclear inclusion bodies were observed in the epithelial cells of these vesicles and ulcers and of adjacent hair follicles and sebaceous glands. There was a mild to moderately severe rhinitis. Keratitis was present in two wallabies. Liver lesions were present in two animals but were unlike those seen in herpesviral hepatitis in other species.

### INTRODUCTION

There are few reports of viral disease in marsupials. A naturally-occurring disease outbreak, in which a herpesvirus was recovered from one of the affected animals, has been described in a small group of Parma wallabies (*Macropus parma*) held in captivity.<sup>1</sup> Signs were fever, conjunctivitis and ano-genital vesicles. Morbidity was high and mortality was moderate. In a few of the fatal cases there were large intranuclear inclusion bodies associated with severe multifocal necrotizing hepatitis and hyperplastic necrotizing bronchiolitis.

The virus recovered in the disease outbreak has been classified as a new member of the herpesvirus family and is considered to have most likely evolved in a marsupial host.<sup>4</sup> Serological evidence suggests that this virus or a closely related herpesvirus is widespread among marsupials in Australia.<sup>3</sup>

This paper describes the lesions produced experimentally in Parma wallabies by the agent.

### MATERIALS AND METHODS

Five Parma wallabies obtained from the wild population of a small island and negative for neutralizing antibodies to Parma wallaby herpesvirus were housed in a 4 m × 4 m isolation pen and held for 10 days prior to exposure to the virus. Wallabies 1 and 2 were given 2 ml of inoculum into a lateral tail vein. Wallaby 3 was given 2 drops of inoculum into each conjunctival sac and 1 drop into each nasal cavity, followed by mild abrasion of these areas by a cotton swab soaked in inoculum. Wallabies 4 and 5 were kept in contact with the inoculated animals. All except wallaby 5 were female, and all were aged about 3 years except for wallaby 1, which was about 1.5 years old. After exposure, the animals were exam-

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ined at least once daily in the following sequence: 1) the two contact animals, 2) the animal given mucosal inoculation, and 3) the two intravenously inoculated animals.

The inoculum was from a frozen virus stock composed of the combined harvests, clarified by centrifugation, of Parma wallaby kidney (WK) and Pretty-faced wallaby (*Macropus parryi*) fibroblast cell cultures, each infected with the virus as the third pass after autoculture. The inoculum, titrated after use, contained  $10^{5.0}$  TCID<sub>50</sub>/ml in WK cells.

Virus recovery was attempted from the head and genital mucous membranes of animals alive at post-exposure (p.e.) days 2 (animal 2 gingiva only), 3, 7, and 11 using phosphate buffered gelatin saline soaked swabs and from citrated blood at p.e. days 2 (animal 2 only), 3, 7, and 10. Lung, liver, spleen, kidney of all animals and peritoneal fluid of animal 4, vaginal mucous of animal 3, and a lymph node of animal 1 collected at necropsy were examined for virus by inoculation of primary WK cell cultures with swab fluid, blood or 10% tissue homogenates.

## RESULTS

All wallabies became infected and the time of onset and rate of progression of signs varied with the route of infection (Table 1).

Swelling of eyelids and upper lips was the initial clinical sign. Occasionally, additional areas of the head such as the lateral margin of the nares or one or both sides of the face were swollen. Small numbers of 1-2 mm vesicles and ulcers appeared on the upper lip skin and mucous membrane and adjacent gingiva. The vesicles ruptured in about 24 h and ulcers formed. Vesicles and ulcers enlarged only slightly on the skin surface and, in two animals, on the upper lip mucosa. On the other hand, in two animals an extensive and rapid increase in size of the lesions on the mucosa of the

TABLE 1. Distribution and severity of lesions in Parma wallabies experimentally infected with herpesvirus.

Wallaby number	Route of infection	Post-exposure day of symptoms	Post-exposure day of death	Lesions Ulcers and Vesicles					Keratitis
				Skin	Upper lip mucosa	Ano-genital skin	Genital	Con-junctiva	
1	intravenous	2	5	+	+++	++	+	+++	
2	intravenous	2	5	+	+	+		+	
3	mucosal	3	7*	+	+++	+		+++	++
4	contact	7	11*	+	+++	++	+	+++	
5	contact	7	11**	+	+	++	+	++	+

\*killed when severely affected

\*\*killed when mildly affected

lip was seen, so that in these, and in a third animal that was severely affected at the onset of illness, a large proportion of the area was involved (Fig. 1).

Twenty-four to 48 h after the appearance of oral vesicles and ulcers all animals had a serous bilateral ocular discharge. In animal 3 the ocular discharge became purulent. Serous nasal discharge occurred in animals 2 and 3.

At necropsy, the vesicles and ulcers of the labial and gingival mucosa were sharply defined. Vesicular fluid volume was small and the fluid was not under pressure. Vesicles ruptured easily when touched. Small and large ulcers had a hyperemic and hemorrhagic floor. Fibrino-necrotic debris accumulated on the surface of large ulcers of the upper lip mucosa and adjacent gingiva.

In the 1 cm wide band of sparsely-furred skin around the ano-genital area of the male and female infected by contact and a female inoculated in-

travenously a few 2-3 mm vesicles and ulcers were observed. The mucosa of both vaginas had a few 2 mm ulcers posteriorly and the preputial mucosa had several 2 mm ulcers near the muco-cutaneous junction.

All animals had some degree of subcutaneous edema of the muzzle, lips and eyelids and over the cranium. Some of the lymph nodes of the head were slightly enlarged. Other lesions were bilateral corneal opacity in animal 3, miliary white foci in the liver of animal 1, and probably traumatically-induced inguinal subcutaneous edema and hemorrhage, unilateral proximal hindlimb myonecrosis and pelvic cavity hematoma in animal 2. Animals 2 and 3 had mild pulmonary edema. Animal 1 had blood-stained intestinal contents, but no source of hemorrhage was found.

At the microscopic level, numerous additional small vesicles and ulcers were recognized in all five animals on the skin



FIGURE 1. Large vesicles in the upper lip mucosa (small arrows) and upper lip skin (large arrow) of wallaby 4 on post-exposure day 9.

of the upper and lower lips, eyelids, anogenital area and the conjunctival, vaginal and preputial mucosa. In one animal (number 4) there was an erosion of the tongue visible microscopically. Large areas of palpebral conjunctival ulceration were present in all wallabies except number 2, in which the affected area was small. These microscopic lesions and the grossly visible small vesicles and small and large ulcers were all of the same type. Various stages could be seen beginning with ballooning of epithelial cells in the basal layer and stratum spinosum and the appearance in a few of these cells of a large basophilic intranuclear inclusion body almost filling the nucleus. The cells of the stratum spinosum underwent acantholysis, leading to the formation of a usually unilocular intra-epidermal vesicle (Fig. 2). This was followed by separation of the

cells of the basal layer. At this time intranuclear inclusion bodies, both large basophilic and smaller eosinophilic with a halo, were numerous. Multinucleate cells, with or without inclusion bodies, were present in some vesicles (Fig. 3). The cells above the vesicle sloughed as the margin of the lesion extended laterally. As erosion progressed to ulceration, moderate numbers of neutrophils and a few macrophages appeared in the lamina propria. Overlying large ulcers there was a plaque of necrotic epithelial cells, fibrin, blood and bacteria.

Sebaceous glands and the upper parts of hair follicles and sinus hair follicles adjacent to skin vesicles and ulcers often had a few necrotic epithelial cells, numerous intranuclear inclusion bodies in epithelial cells and a mild infiltration of neutrophils. This reaction was notable in the large sebaceous glands associated

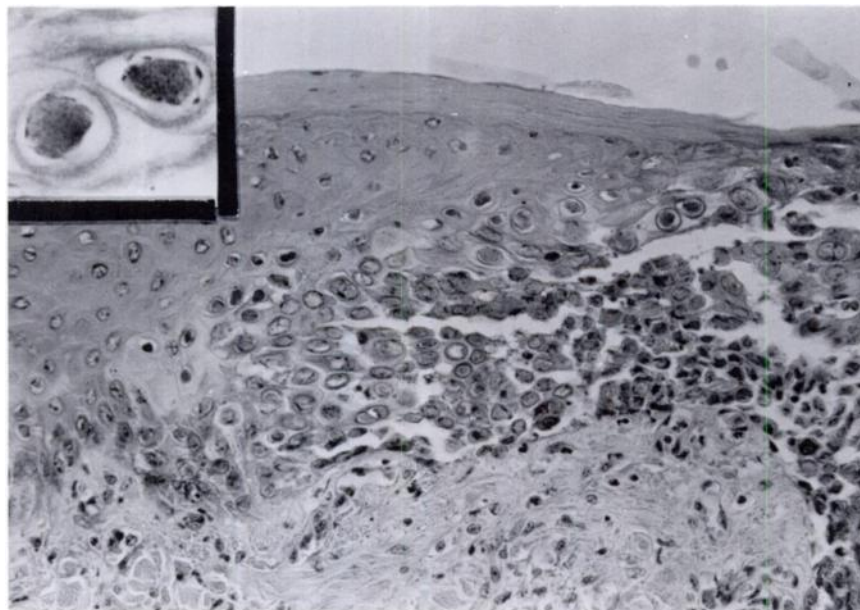
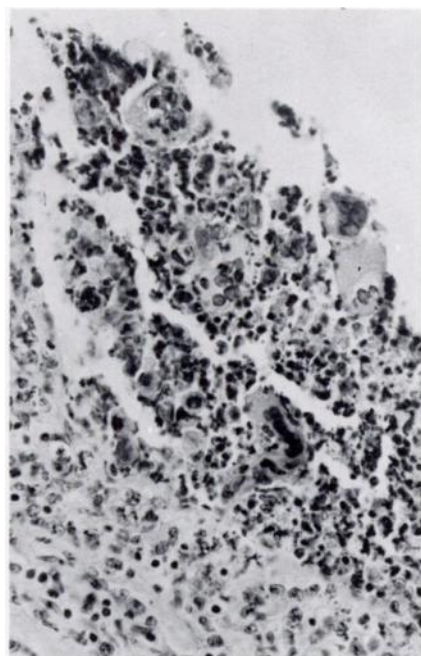
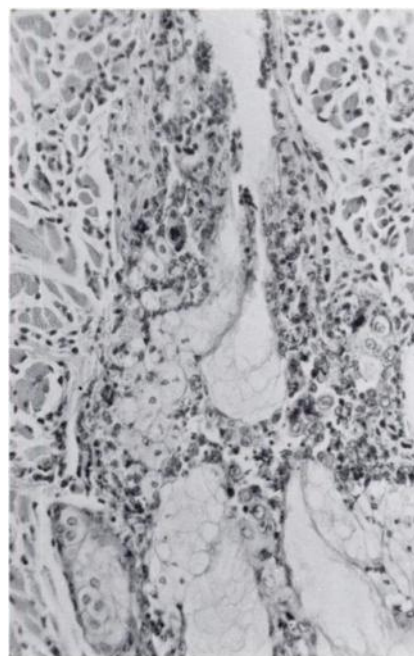


FIGURE 2. Edge of intra-epidermal vesicle in the lip mucosa of wallaby 2. Cells of basal layer and stratum spinosum are swollen and acantholytic. Numerous intranuclear inclusion bodies are present. Inset shows inclusion bodies almost filling the nuclei of 2 rounded epithelial cells.



**FIGURE 3.** Multinucleate cells, some with inclusion bodies in a vesicle of ano-genital skin of wallaby 4.



**FIGURE 4.** Acute inflammation of sebaceous gland of ano-genital skin of wallaby 4.

with the sinus hair follicles of the lips and eyelids and particularly the ano-genital skin (Fig. 4). The inner dermal sheath of the upper part of the sinus hair follicles was often infiltrated by neutrophils and had intranuclear inclusions. Often there were vesicles and ulcers in the skin and mucous membrane of the eyelid margins, and then the tarsal glands had severe lesions similar to those in sebaceous glands.

Keratitis was present in two animals. In wallaby 3, whose conjunctival sacs were inoculated, it was bilateral, with corneal edema, small numbers of neutrophils in the corneal propria and moderately severe ulceration. In wallaby 5 the keratitis was unilateral, with mild neovascularization, extensive multifocal ulceration and a few intranuclear inclusion bodies in the epithelium.

Most mesenteric and parotid lymph nodes were reactive, with prominent germinal centers. In some nodes there was pyknosis and phagocytosis of a few scattered lymphocytes. Spleens were normal.

Livers were abnormal in two animals. Wallaby 1 had numerous small areas of subacute necrotizing hepatitis that were heavily mineralized. Inclusion bodies were not present. In wallaby 2 there was moderate bile ductule proliferation and moderately severe multifocal subacute hepatitis. The foci were intrasinusoidal collections of lymphocytes and macrophages. Hepatocellular necrosis was not present nor were inclusion bodies.

Nasal turbinates of all except number 4 were examined. There was a mild to moderately severe acute rhinitis with

multifocal erosion or ulceration and fibrinous exudation. Intranuclear inclusions were prominent in respiratory epithelial cells of animals 1 and 2 and in cells of the lamina propria of animal 2. Pulmonary edema was mild in animals 1 and 3, moderately severe in animal 2. Mild diffuse subacute interstitial pneumonia was present in wallaby 4 and very mild acute bronchiolitis in wallaby 5.

The adrenal of wallaby 5 had mild multifocal hemorrhage and necrosis in the zona fasciculata. Adrenals of animals 1 and 3 were normal and those of animals 2 and 4 were not examined.

The only lesion of the gastrointestinal tracts was moderately severe acute focal ulcerative gastritis in the pyloric area of animal 4.

Other organs examined histologically and found to be normal were penis, testis, trachea, salivary gland, esophagus, gall bladder, pancreas, small and large intestine, kidney, urinary bladder, heart, skeletal muscle, thyroid, brain and pouch.

The virus was recovered from 6 of 53 clinical specimens and from 1 of 25 postmortem specimens. All clinically sampled sites except reproductive tract and all animals except number 1 yielded virus at some stage. Thus virus was recovered from gingiva of animal 2 on p.i. day 2, blood of animal 2 on p.i. day 3, conjunctiva of animal 3 on p.i. day 7, conjunctiva of animal 4 on p.e. day 9 and conjunctiva and nasal mucosa of animal 5 on p.e. day 11. The only postmortem sample from which virus was recovered was lung of animal 5. Titers of virus in swab fluids were in the range  $10^1$ - $10^3$  TCID<sub>50</sub>/ml, while the blood of animal 2 contained  $10^2$  TCID<sub>50</sub>/ml and the lung of animal 5 contained  $10^1$  TCID<sub>50</sub>/g virus.

## DISCUSSION

The disease produced can be attributed to the herpesvirus used for challenge

because the agent could be recovered from affected animals and the lesions had the general pattern of those caused by herpesviruses. Thus the recoveries of virus from a gingival swab of one of the intravenously inoculated animals at p.i. day 2 and from the blood of this animal at p.i. day 3 and from the conjunctiva of one of the contact animals at 9 days p.e. and from the conjunctiva and nasal cavity and lung of the other contact animal at 11 days p.e. are confirmative evidence. The time interval of 7 days between inoculation into and recovery from the conjunctival sac of one animal suggests that this environment is suitable for viral persistence and probably replication. The lesions involved the skin, cornea, conjunctiva, oral cavity, upper respiratory tract and lower genital tract and were very similar to those of the skin and mucosal surfaces in various herpesviral diseases of man and other primates.<sup>2</sup>

Experimental and naturally-occurring Parma wallaby herpesvirus infections differ from one another in their morbidity and mortality and in the distribution of their lesions. In the initial disease outbreak,<sup>1</sup> 16 of the group of 20 were affected and only 7 died; however in the experimental group of five, two died and two were killed when moribund. While the fifth was only mildly affected when killed, he was probably in the early stage of a fatal disease process. Although multifocal necrotizing hepatitis similar to that found in many fatal herpesviral diseases was observed in some of the field cases, the two abnormal livers of the experimental animals did not have typical herpetic lesions in that one had very heavily mineralized necrotic foci and the other had granulomatous disease probably caused by some other agent. Ulceration of the lip mucosa, a prominent feature of the experimental disease, was not noted in the field cases, but it could have been overlooked.

The lesions in this fatal experimental disease were almost restricted to the skin



and mucous membranes. Most herpesviral diseases with this lesion distribution are non-fatal. Examples are herpes simplex virus infection in man, herpes B virus infection in rhesus monkeys, herpes T virus infection in squirrel monkeys, equine herpesvirus 1 and 3 infections, infectious bovine rhinotracheitis and feline viral rhinotracheitis. The respective viruses do cause fatal disease, but generally only in fetuses or neonates of the primary host species or in a secondary host species. Then the lesions are visceral or neural. A few herpesviral diseases, such as herpes T virus infection of owl monkeys and marmosets and herpes simplex virus infection in owl monkeys, have epithelial as well as generalized lesions, and these diseases are fatal.

The serological evidence for this virus being widespread in several species of

Australian marsupial<sup>1</sup> and the properties of the virus<sup>1</sup> suggest that the Parma wallaby, in having fatal disease, is a secondary host, and that the primary host, which would have an inapparent or mild recurrent disease, is another marsupial.

The route of experimental infection influenced the time of onset and rate of progression of clinical signs, but the lesions were similar for all three methods. Several means of transmission to the two contact animals were possible. Abrasion of skin and mucous membranes by contaminated woodshaving bedding could have occurred. Inhalation would have been a possible method as the pen was dusty because the animals hopped around very actively. As the level of sexual activity in the pen was high, venereal transmission could have occurred.

#### Acknowledgement

I wish to thank E. Batty for the virology.

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*Received for publication 14 October 1980*