



NOTE

Pathology

A case of malignant melanoma, a possible primary site in the digit, with systemic metastasis in a mini-Rex

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ABSTRACT. We report a case of systemic metastasis of malignant melanoma in a mini-Rex (*Oryctolagus cuniculus*). The animal presented with lameness of the right hind limb, swelling of popliteal lymph node, and a black mass on the first digit. Paralysis of hindlimbs and forelimbs, dysuria, and dysphagia progressed over time, and the rabbit died on day 35 from the first visit. At necropsy, many black lesions were observed in multiple organs including the marrow of most bones. Histopathologically, the tumor cells had highly atypical nuclei of various sizes and an abundant eosinophilic cytoplasm, and some cells contained melanin granules. These cells were positive for PNL2 and S-100, melanoma markers, by immunohistochemistry. This is the first report of malignant melanoma in a mini-Rex with severe malignancy and systemic metastasis including the bone marrow.

KEYWORDS: bone marrow, digit, malignant melanoma, mini-Rex, rabbit

J. Vet. Med. Sci.

84(12): 1574–1578, 2022

doi: 10.1292/jvms.22-0181

Received: 14 April 2022

Accepted: 30 September 2022

Advanced Epub:

12 October 2022

In rabbits, malignant melanoma (MM) is uncommon, and tends to occur in the auricle, eyelid, head, extremities or groin [2, 6, 18, 20, 22]. Several cases of MM in rabbits are recorded including distal metastasis in some organs, owing to the limited number of cases, prognosis, metastasis, and a breed predisposition of MM in rabbits are unclear [2, 6, 18, 20, 22]. Here, we describe the first case of a mini-Rex (*Oryctolagus cuniculus*) that presented with progressive clinical symptoms including lameness, dysuria, and dysphagia, and was diagnosed with MM. In our case, the digit of right hind limb was possible primary site and metastases to various organs including the bone marrow were observed.

A 5-year old mini-Rex castrated male weighing 2.0 kg presented to the referring veterinarian with lameness of the right hind limb. On the first visit, a general physical examination revealed a mild decrease in general body condition (body condition score 2/5, according to previous study [2, 14]) with swelling of popliteal lymph node (20 × 15 × 10 mm) and a 3-month history of a black and small nodule (15 × 10 × 10 mm) on the digit of the right hind limb with some pain in the affected area. A postural reaction test showed decreased reactivity in the right hind limb. Radiographic examination in ventral-dorsal position and right-lateral position without anesthesia showed no abnormalities in the pelvis and spine. Because bacterial infection or encephalitozoonosis were suspected, 10 mg/kg enrofloxacin (Baytril[®], Bayer, Leverkusen, Germany) was orally administered twice a day, and 10 mg/kg fenbendazole (Panacur[®], MERCK, Vienna, Austria) was orally administered once a day for 7 days. On day 2, the rabbit had lameness of the left hind limb, decreased reactivity in the postural reaction test, and paralysis of the right hind limb. A reaction to noxious stimuli was present in the left hind limb but absent in the right hind limb. In addition, 0.2 mg/kg meloxicam (Metacam, Boehringer Ingelheim, Ingelheim, Germany) was orally administered once a day for 5 days for analgesia. However, no improvement in clinical symptoms was observed after continued administration. The rabbit then developed intermittent paralysis that progressed from hindlimbs to forelimbs. On day 15, a black cutaneous mass (2 mm diameter) was detected on the right abdominal skin as well as dysuria. Compression urination was performed twice a day, and the animal received subcutaneous infusion of Lactated Ringer's solution (60 mL/day) (Solulact[®], Terumo, Tokyo, Japan) once every 2 days. On day 28, the postural reaction test showed decreased reactivity in the right forelimb, and a black mass was observed on the left eyelid. On day 32, the animal showed severe paralysis of both forelimbs, and dysphagia, respiratory difficulty and died on day 35.

A necropsy was performed with a consent of the owner. Many black masses were observed in several organs including the lungs,

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heart, liver, spleen, stomach, kidneys, adrenal glands, pituitary gland, trachea, lymph nodes, skeletal muscles, and bone marrow (Fig. 1). Most masses were smaller than 5 mm, but some masses were bigger, including those in the lung (8 mm), right hindlimb digit (15 × 10 × 10 mm), and right popliteal lymph node (25 × 17 × 13 mm). The masses had displaced approximately 50% of the lungs, and the marrow of bones (ribs, long bones of extremities, vertebra and skull) was mostly deep blackish. A black mass, 1.5 cm in diameter, was present on the palmar side of the first digit of the right hind limb (Fig. 2A). The surface of the mass was alopecia and mildly ulcerated at the center. The cut surface of the mass was black and somewhat fragile, the border between the mass and the bones (distal and middle phalanges) were well defined. Wright-Giemsa-stained stamp preparation of the mass lesion showed a cluster of atypical cells with a high N/C ratio, as well as countless brownish granules in the background (Fig. 2B). The nuclei were round to ovoid with coarsely stippled chromatin and shaped nucleoli. Occasionally, cells with granules in their cytoplasm were observed, which thought to be melanin-producing cells or melanin-laden macrophages.

Histopathologically, the black masses in several organs were composed of proliferating neoplastic cells (Fig. 3). The cells were arranged in sheets that were partly capsulated in collagen fiber and showed polygonal to spindle shape. The nuclei were highly atypical with anisokaryosis and many mitoses (average of three images in 400× magnification). Some cells contained melanin granules in the cytoplasm. Many melanin-phagocytic macrophages were also observed. Similar neoplastic cells were observed in the blackish bone marrow, liver, spleen, kidneys, heart, trachea, lungs, stomach, adrenal glands, pituitary gland, conjunctiva, skeletal muscles of right forelimb and intra-abdominal fat tissues. The foci of neoplastic cells in these organs were small (less than 5 mm other than lungs) and accompanied by melanophages. The marrow cavity of the right humerus was almostly replaced by the proliferation

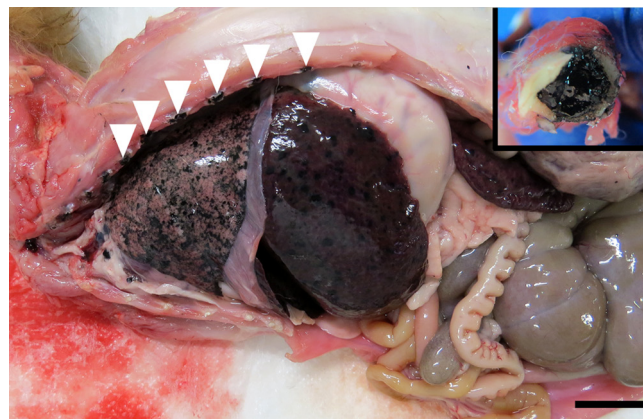


Fig. 1. Appearance of the organs in body cavities at necropsy. Small black foci are found in lungs, liver and spleen. Note the cross-section of rib bones (white arrowheads) are black. The insertion shows an appearance of cross-section of diaphyseal region of right femur. Bar, 2 cm.

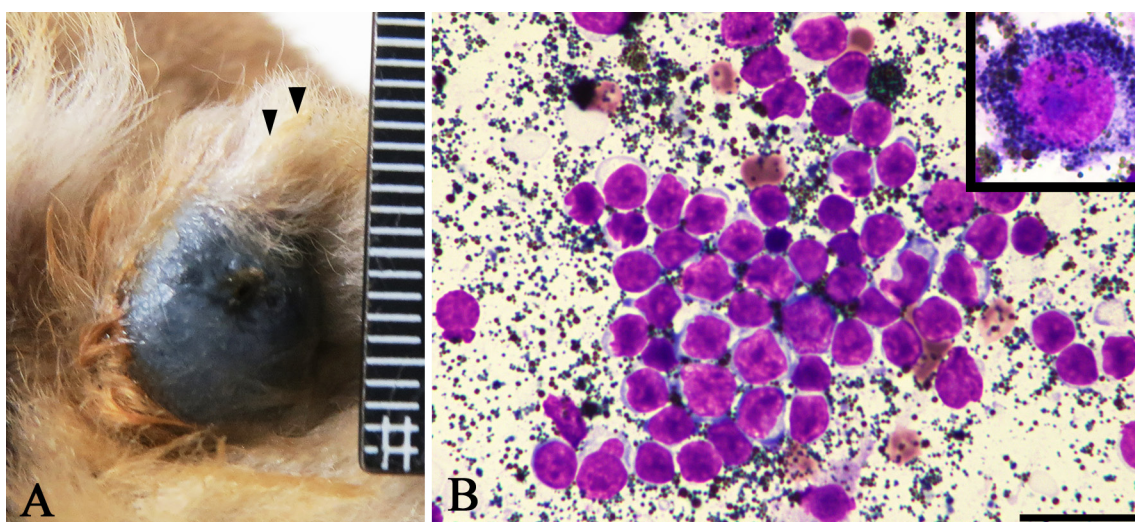


Fig. 2. Gross appearance of the digital mass in the right hind limb (A). The mass is blackish and the surface is alopecic, mild ulceration at the center. Arrowheads indicate the nail. Scale marks, 1 mm intervals. Cytology of the stamp preparation of the right digital lesion (B). Cluster of atypical cells with high N/C ratio and myriad brownish granules. Inset shows the cell with granules within the cytoplasm. Wright-Giemsa stain. Bar, 20 µm.

of neoplastic cells and melanophages. In both kidneys, focal fibrosis with the infiltration of mononuclear cells, heterophils and melanophages was located in subscapular cortex, which was speculated to be a micro-infarction caused by tumor emboli. These findings suggested that the neoplastic cells spread mainly hematogenously to various organs, and also partly via lymphatics, in the present case. Immunohistochemistry (IHC) for melanoma markers, PNL2 (Santa Cruz Biotechnology, Santa Cruz, CA, USA), Melan-A (Dako, Glostrup, Denmark) and S-100 (Dako), those are useful for the diagnosis of rabbit's MM [18], was performed. Briefly, deparaffinized sections were microwaved in 0.01 M citrate buffer (pH 5.4) and then treated with 3% hydrogen peroxide. After incubation with blocking reagent (Nacalai Tesque, Kyoto, Japan), the sections were reacted with each primary antibody (1:150 for PNL2, 1:50 for Melan-A, 1:300 for S-100, respective fold dilution) overnight at 4°C. Then the sections were treated with peroxidase-conjugated secondary antibodies, the positive signals were visualized 3, 3'-deaminobenzine and nuclear counter stained with haematoxylin. About 25% of neoplastic cells showed positive for PNL2 (Fig. 4). The most neoplastic cells showed weakly positive for S-100, while negative for Melan-A. Based on these findings, a diagnosis of MM, and its systemic metastases were made.

Initially, the digital lesion of the right hind limb of the present case was suspected to be trauma and/or bacterial infection; however, antibiotics, antiparasitics, and nonsteroidal anti-inflammatory drugs were not effective. Although black masses were found on the skin and left eyelid, spinal cord diseases such as progressive myelomalacia or meningeal tumor were considered because the notable clinical signs were mainly lameness and paralysis. Postmortem and histopathological examination revealed systemic metastasis of MM. Although MM is not common in rabbits, 13 cases were previously documented [2, 6, 18, 20, 22] and are summarized in Table 1. Most cases of MM in rabbits occur in males (10/13 cases) and in animals >3 years of age, consistent with the present case. Although a breed predisposition of MM in rabbits has not been documented, to the best of our knowledge, this is the first report of MM in a mini-Rex.

The owner reported that the black nodule on the right first digit had been present for approximately 3 months. The right popliteal

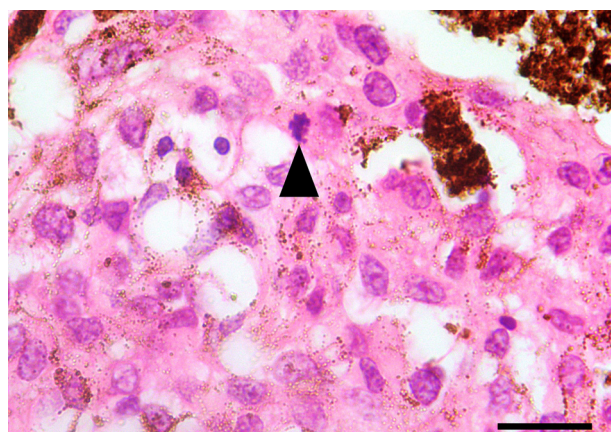


Fig. 3. Histopathology of right popliteal lymph node. Proliferation of neoplastic cells those have small granules in their cytoplasm and melanophages, suggesting metastasis of malignant melanoma from digital lesion. Arrowhead indicates the mitosis of the neoplastic cells. HE. Bar, 20 µm.

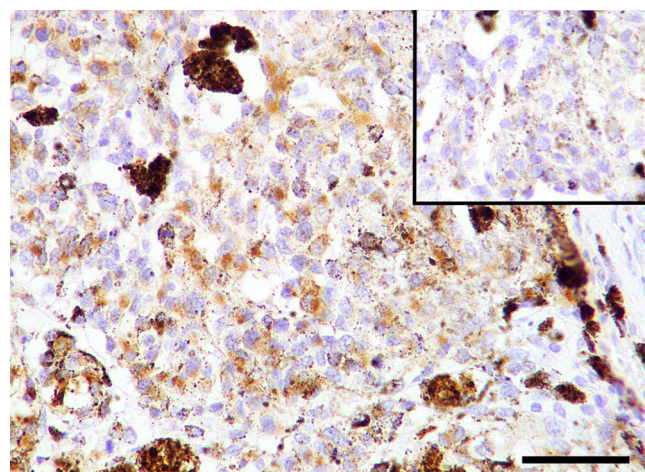


Fig. 4. Immunohistochemistry for a melanoma marker PNL2 in right popliteal lymph node. Some of the neoplastic cells show positive signals in their cytoplasm (brownish color). Nuclear counterstained by hematoxylin. Bar, 50 µm. Inset is a view from serial section of the negative control (using PBS instead of the primary antibody).

Table 1. Summary and comparison of the cases of cutaneous malignant melanoma in rabbits

Cases	Breed	Sex (number)				Age (years)	Primary site	Metastasized lesion	Ref
		M	CM	F	SF				
1	Mix				1	7–10 (estimate)	Skin (nasal canthus)	ND	[2]
2	Dwarf rabbit		1			4	Skin (inguinal region)	Parietal pleural, lungs, liver	[6]
3	Mix		1			4	Skin (scrotum)	Lungs, liver, spleen, kidneys	[18]
4	Mix		1			10	Eyelid	Lungs, liver, spleen, kidneys	[18]
5–12	-	5	1	2	0	2–8 (3.8*)	Ear pinna, eyelid, head, perivulvar haired skin, scrotum, thigh, stifle	ND	[20]
13	NZW		1			3.5	Skin (face)	Parietal pleura, diaphragm	[22]
14	Mini-Rex		1			5	Digit (hind limb)	Lungs, heart, liver, spleen, stomach, kidneys, adrenal glands, pituitary gland, trachea, lymph nodes, skeletal muscles, bone marrow	Present case

M: intact male, CM: castrated male, F: intact female, SF: spayed female, NZW: New Zealand white rabbit, ND: not mentioned in the report. *: median age of 8 rabbits, range from 2 to 8 (in years). Data from a retrospective study [20].

lymph node metastasis was the largest lesion among metastatic foci. Thus, the primary lesion was suspected to be a digit on the right hindlimb. MM in rabbits has been reported on eye pinna, eyelid, head, skin of the inguinal region, perivulvar haired skin, scrotum, thigh, and stifle. In previous cases, metastasis of MM was observed in the lungs, parietal pleura, diaphragm, liver, and spleen (Table 1) [6, 18, 20, 22]. In the present case, it was suspected that the primary lesion of MM was the digit, and metastases were observed in various organs that had not been reported previously in rabbits including the heart, stomach, adrenal glands, pituitary gland, trachea, lymph nodes, skeletal muscles, and bone marrow. In addition to the characteristic findings, a myriad small metastatic foci (1–3 mm) that did not form obvious nodules was observed. This strongly suggests that MM on the digit in rabbits can spread to these organs through blood and lymphatic vessels. The most characteristic finding of the present case was systemic metastasis to the bone marrow. Only a few cases of MM metastasizing to the bone marrow have been reported in animals [9, 13]. There are no reports of MM metastasized to the bone marrow in rabbits in the veterinary literature. This is the first report of MM with bone marrow metastasis in rabbits. The MM in the present case may have shown a more aggressive behavior than those reported previously, and digital MM in rabbits might be more malignant than other cutaneous MMs, such as those in dogs [12].

Clinical symptoms such as lameness, dysuria, and dysphagia were observed in the present case. One of the characteristic symptoms of this case was lameness. The case had multiple metastases to skeletal muscles and bone marrow, which was assumed to be the cause of the multiple limb lameness. Lameness in dogs of MM is also observed, but usually only in the affected limb or the limb with metastatic lesions. Therefore, we considered lameness to be an important finding reflecting the high malignancy of MM in the present case. In humans, infiltration of MM into the bladder, urethra, uterus, or prostate, causes dysuria [4, 19], and infiltration of MM into the esophagus causes dysphagia [10, 15]. In dogs, there were reported that infiltration of MM into colon caused dysuria [17]. However, in the present case, metastasis to these organs was not observed in the histopathological examination. On the other hand, the clinical symptoms such as dysuria, and dysphagia may have been caused by infiltration of MM into the brain or spinal cord, because melanoma was present in the pituitary gland in the current case. However, infiltration of neoplastic melanocytes was not observed in the brain parenchyma and spinal cord. These findings support that the lameness and paralytic signs in the present case were caused by damage to peripheral tissues including skeletal muscles and bones, and not by involvement of the central nervous system.

By IHC, the neoplastic cells were positive for PNL2, negative for Melan-A. There are some reports on investigation of multiple melanocyte markers for diagnose of MM in humans [3], dogs [16] and also rabbits [18]. In dogs, it is reported that sensitivity and specificity of Melan-A for the detection of canine oral amelanotic melanocytic neoplasms were 81.6% and 100%, and these of PNL2 were 89.8% and 100% [16]. A few reports on melanocyte markers used for IHC of MM in rabbits, showed that 2 of 2 MM in rabbits were positive for PNL2, and 4 of 4 MM in rabbits were positive for Melan-A [2, 18, 22]. The negative reaction of Melan-A in our case suggests the sensitivity of Melan-A for the detection of MM in rabbits might not be 100%, as those reported in dogs [16]. Therefore, to use multiple melanocyte markers for diagnosis of melanocytic tumors in rabbits, are recommended.

The mini-Rex is a breed of domestic rabbit, and the number of mini-Rex kept as pets has been increasing. Cutaneous neoplasms or cutaneous squamous cell carcinomas, mammary tumors, herpesvirus infection, and gastrointestinal stasis have been reported in pet rabbits [1, 7, 8, 11, 20], although there are a few case reports about diseases of mini-Rex, such as polypoid cystitis or ossifying fibroma [5, 21]. Reports of tumors in mini-Rex are also rare [1, 11], and it remains unclear whether the mini-Rex is a breed with a high incidence of tumors including MM. The present case indicates that elderly mini-Rex might develop MM and that it might show highly aggressive behavior. The treatment of rabbits with MM is difficult because there are no studies on the prognosis or effective chemotherapy for pet rabbits, and local recurrence within a few months after surgery has been reported [20, 22]. Further studies on MM are necessary to improve our knowledge and control MM in rabbits.

CONFLICTS OF INTEREST. The authors declare no potential conflicts of interest with respect to the publication of this manuscript.

ACKNOWLEDGMENT. This work was supported by research basic expenses of Tottori University.

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