Embryonal rhabdomyosarcoma in a Siberian chipmunk (*Tamias sibiricus*): a case report

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ABSTRACT: A 2-year-old female Siberian chipmunk (*Tamias sibiricus*) was presented to the veterinary clinic for swelling, pain and lameness of the left rear leg. Radiologically, an invasive tumour around the distal femur was suspected, and the leg was surgically amputated and submitted for histopathological diagnosis. Microscopically, the mass was densely packed with multinucleated strap cells that had round-to-oval, or elongated nuclei with prominent nucleoli. These neoplastic cells occasionally formed myotubes with cross-striations and were immunohistochemically positive for muscle markers including desmin and myogenin. Consequently, embryonal rhabdomyosaroma myotubular variant of the leg with metastasis to the femur was diagnosed. Spontaneous rhabdomyosaromas are rare tumours in animals and humans, and this is the first report of its occurrence in a Siberian chipmunk.

Keywords: myogenin; PTAH stain; rodents; skeletal muscle; squirrel

Rhabdomyosarcomas (RMSs) are rare malignant tumours of the striated muscle cells in animals and humans. In domestic animals, case reports of RMSs in dogs are more common than for any other species, followed by reports in cats, horses, sheep and pigs (Caserto 2013). RMSs are also considered the rarest naturally occurring tumours in rodents and have been primarily reported in laboratory rats and mice (Table 1). In contrast to reports describing the frequent occurrence of RMSs in younger dogs (commonly less than or equal to 2 years old) and children (Caserto 2013), based on the information of Merck Research Laboratories, older rats were considered to have a higher incidence of RMSs (an average age of 103 weeks; Conner 1994). However, the relationship to age is controversial, because there are more reported cases from younger rats and mice in recent times (commonly less than or equal to 1-year-old, the youngest was eight weeks old; Sundberg et al. 1991; Conner 1994; Germann et al. 1994; Radi 2006; Chang et al. 2008) and A/J

mice show a high frequency of RMSs in all age groups (Sher et al. 2011). Therefore, more studies are needed to definitively characterise RMSs in rodents. The Siberian chipmunk (*Tamias sibiricus*) is a member of the family Sciuridae within the order Rodentia that primarily inhabits Northeastern Asia and has been raised as a pet. There are a few reports of neoplastic diseases in squirrels (Trigo and Riser 1981; Shivaprasad et al. 1984; Fukui et al. 2002; Honnold et al. 2007; Tamaizumi et al. 2007; He et al. 2009; Oohashi et al. 2009; Panakova et al. 2010; Carminato et al. 2012; Childs-Sanford et al. 2015); however, RMSs have not been reported. Here we provide the first report of a RMS in a Siberian chipmunk together with histopathological findings.

Case description

A 2-year-old intact female Siberian chipmunk, which was raised as a pet, presented to the vet-

Table 1. Previously reported spontaneous rhabdomyosaromas (RMSs) in rats and mice

Species	Breed	Location	Age of onset	Type of RMSs	No. of animals reported (incidence rate)	Reference(s)
Rats	Sprague-Dawley (SD)	thoracic and abdominal cavities, genu, ear	8 weeks to 1 year	embryonal N/A	4	Conner 1994; Germann et al. 1994; Radi 2006; Chang et al. 2008
	Lewis	uterus	25 months	N/A	1	Kaspareit-Rittinghausen and Deerberg 1990
	Wistar	thoracic and abdominal cavities	67 week	pleomorphic	1	Kerry et al. 1995
	Fisher 344	skeletal muscle	N/A	N/A	3 (< 0.1%)	Haseman et al. 1998
Mice	A/J	skeletal muscle	6 months to $\geqq$ 20 months	pleomorphic	35 (27.5%)	Sher et al. 2011
	BALB/c	legs, abdominal wall	2–8 months	N/A	10 (< 0.01%)	Sundberg et al. 1991
	B6C3F	skeletal muscle	N/A	N/A	1 (< 0.1%)	Haseman et al. 1998

N/A = not applicable

erinary clinic for swelling, pain and lameness in the left rear leg. On radiographic analysis, a focal radiolucent lesion with bone lysis was detected in the distal area of the left femur, and a malignant tumour was strongly suspected (Figure 1). The leg was amputated by surgery and submitted for histopathological diagnosis. The muscles around the stifle joint and below were much larger and harder than normal. These tissues were immersed in 10% neutral buffered formalin and processed

Figure 1. Dorsoventral radiographic view of a Siberian chipmunk. A focal radiolucent lesion caused by bone lysis in the distal area of the left femur (dotted circle)

routinely for embedding in paraffin wax, then cut into 4 µm thick sections, which were subsequently stained with haematoxylin and eosin (H&E), and phosphotungstic acid-haematoxylin (PTAH). Anti-desmin (Santa Cruz, USA) and myogenin (DSHB, USA) antibodies were used for immunohistochemistry (IHC). The antigen-antibody complexes were visualised by an avidin-biotin peroxidase complex solution using an ABC kit (Vector Laboratories, USA). Sections were developed with 3,3'-diaminobenzidine and finally counter-stained with Mayer's haematoxylin.

Microscopically, basophilic neoplastic cells appeared without any circumscription or encapsulation and infiltrated into adjacent normal muscle fibre (Figure 2A). Densely packed multinucleated strap cells with minimal-to-abundant eosinophilic cytoplasm were evident. These neoplastic cells had round-to-oval or elongated nuclei with prominent nucleoli and occasionally formed myotubes with cross-striations that were identified by PTAH staining (Figures 2B and 2C). Overall, 2–3 mitotic figures per high power field were detected. Small round-to-stellate or spindle-shaped cells and long myotubular fibres showing cross-striations embedded in abundant myxoid matrix were observed in some areas of the tumour (Figure 2D). The neoplastic cells were immunohistochemically positive for muscle markers including desmin (Figure 3A) and myogenin (Figure 3B). Spindle cell infiltration with destruction of bone was observed in the distal area of the left femur. Consequently, the neoplasm was diagnosed as a myotubular variant of embryonal RMS.
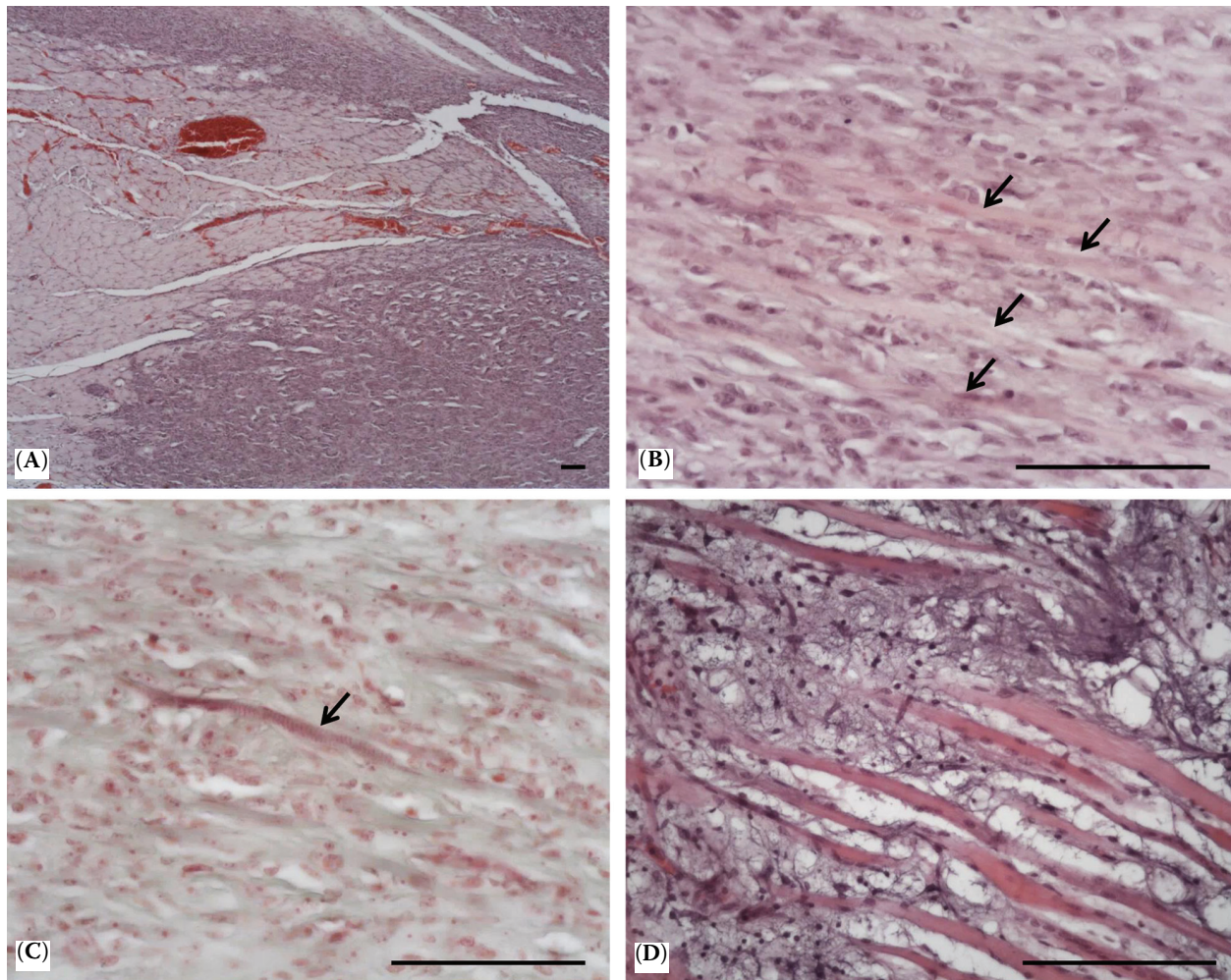
Figure 2. Microscopical examination of the rhabdomyosaroma in the left rear leg of the Siberian chipmunk. (**A**) Densely packed basophilic cells (top and bottom) infiltrate into adjacent normal muscle fibres (middle). (**B**) Multi-nucleated strap cells with round-to-oval or elongated nuclei and eosinophilic cytoplasm are visible. These cells occasionally form myotubes (arrows). (**C**) The cross-striations (arrow) of myotubes are identified by phosphotungstic acid-haematoxylin staining. (**D**) Small round-to-stellate or spindle-shaped cells and long myotubular fibres embedded in abundant myxoid matrix. H&E (**A**, **B**, **D**), phosphotungstic acid-haematoxylin (**C**), scale = 100 μm

DISCUSSION AND CONCLUSIONS

Generally, RMSs are categorised into embryonal, botryoid, alveolar and pleomorphic types based on histopathological features, and embryonal RMSs are further divided into myotubular and rhabdo-myoblastic variants on the basis of the dominant cell morphology (Caserto 2013). IHC is an important tool for diagnosis of RMSs. Vimentin, desmin, actin, myosin, myoglobin, myo D1 and myogenin are commonly used markers, which show differing expression depending on the differentiation of the neoplastic cells (Honnold et al. 2007; Caserto 2013). RMSs can be diagnosed by detection of one or more muscle-specific markers without expression of smooth muscle markers in IHC (Caserto 2013). Desmin is expressed in skeletal, cardiac, smooth muscle cells and myofibroblasts. Myogenin is an embryonic transcription factors expressed during myotube maturation and has been used as a specific and sensitive marker for RMSs. Undifferentiated myoblast cells are expected to express less desmin and more myogenin; moreover, myogenin has been found to be expressed diffusely in human alveolar RMSs, which are poorly differentiated tumours. Conversely, only few cells in human embryonal RMSs express myogenin (Hostein et al. 2004; Caserto 2013). Although the sub-classification of
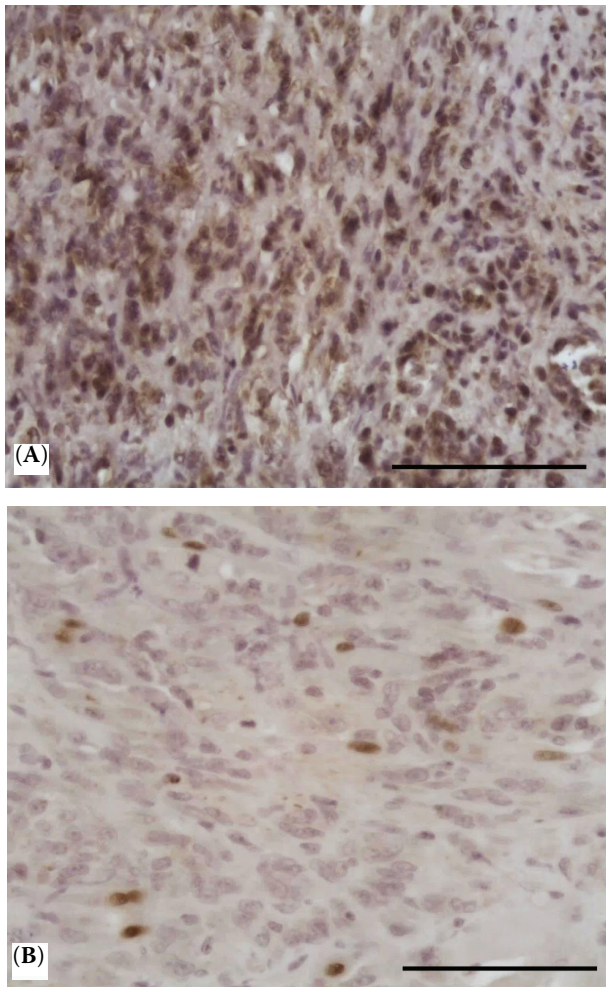
Figure 3. Immunohistochemistry of muscle markers in the RMS of the Siberian chipmunk. (**A**) Most neoplastic cells are positive to desmin. (**B**) A few cells express myogenin in their nuclei. Mayer's haematoxylin counterstain, scale = 100 μm

have not yet been reported. Moreover, only mammary adenocarcinoma (Oohashi et al. 2009) and tail root osteosarcoma (Tamaizumi et al. 2007) have been reported in Siberian chipmunks, and both cases involved animals that were 8-year-old. The present case involved a 2-year-old Siberian chipmunk which is considered to be relatively young because the lifespans of these animals range from five to ten years. To conclude, in this case report, we diagnosed, on the basis of microscopical and immunohistochemical examinations, a myotubular variant of embryonal RMS spontaneously occurring in a young Siberian chipmunk.

RMSs based on expression of IHC markers is not currently possible in veterinary medicine due to the limited number of cases, our case can be classified as a myotubular variant of embryonal RMS that showed diffuse expression of desmin with a few myogenin-positive cells.

There have been a few reports of naturally occurring tumours in squirrels, including mammary tumour (Shivaprasad et al. 1984; Oohashi et al. 2009), osteosarcoma (Tamaizumi et al. 2007), lymphoma (Honnold et al. 2007; Panakova et al. 2010), melanoma (Fukui et al. 2002), mast cell tumour (He et al. 2009), squamous cell carcinoma (Trigo and Riser 1981), transitional cell carcinoma (Childs-Sanford et al. 2015) and adenocarcinoma (Carminato et al. 2012). However, to the best of our knowledge, RMSs

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