JAVMA

Excisional biopsy and radiotherapy for management of an olfactory neuroblastoma in an axolotl (*Ambystoma mexicanum*)

Florent Modesto, DVM[1*]; Alexandra Nicolier, DVM[2]; Clémence Hurtrel, DVM[1]; Jérôme Benoît, DVM[3]

[1]Exotic Pets Department, My Exotic Vet SRL, Clinique Vétérinaire Brasseur, Manage, Belgium
[2]Vet Diagnostics, Charbonnières-les-Bains, France
[3]Department of Radiotherapy, Oncovet Veterinary Clinic, Villeneuve-d'Ascq, France

*Corresponding author: Dr. Modesto (florent.modesto@gmail.com)

https://doi.org/10.2460/javma.20.09.0498

CASE DESCRIPTION
A 4-year-old sexually intact male leucistic axolotl (*Ambystoma mexicanum*) was presented with a 2-week history of dysrexia and difficulty swallowing.

CLINICAL FINDINGS
Physical examination revealed a 1-cm-diameter intraoral mass on the rostral aspect of the palate and swelling of the left nasal fossa. Local invasion into the left nasal fossa was suspected during oral examination. The lesion was marginally excised, and an incompletely excised olfactory neuroblastoma was diagnosed histologically. Five weeks later, physical examination revealed persistent erythema, delayed healing of the rostral portion of the palate, and a mild facial deformity associated with a white mass in the nasal cavity.

TREATMENT AND OUTCOME
6 weeks after excision, adjuvant electron (6-MeV) beam radiotherapy was initiated for treatment of the incompletely excised olfactory neuroblastoma and likely presence of a recurrent mass. The protocol consisted of 4 weekly fractions of 8 Gy each (total, 32 Gy) with the axolotl under anesthesia. No acute adverse radiation effects were noted following radiotherapy. The oral erythema resolved after the third session. No recurrence was observed 2 months after treatment, and the owners reported no abnormal signs at home. The axolotl died 3.5 months after radiotherapy was completed (8 months after marginal excision of the tumor) secondary to an environmental management failure. Postmortem histologic evaluation showed no evidence of neoplasia.

CLINICAL RELEVANCE
In axolotls, olfactory neuroblastoma should be considered in the differential diagnosis of intraoral palatal masses. This report describes the first application of radiotherapy for treatment of an olfactory neuroblastoma in an axolotl.

A 4-year-old 239-g sexually intact male leucistic axolotl (*Ambystoma mexicanum*) was presented with a 2-week history of dysrexia and difficulty swallowing. The owners reported the presence of a lesion in the oral cavity but had been unable to determine its origin. The animal lived with 2 other axolotls in a glass tank (120 X 40 X 50 cm; water temperature, 18 to 21 °C) with an external filter and substrate consisting of Rhine river sand. A third of the water volume was replaced weekly. The diet consisted of axolotl pellets (Aquaterratec; Norgard Ambrock) supplied once or twice a week, with an additional piece of chicken heart or trout every 2 months. No pertinent medical history was reported for any of the axolotls.

On physical examination of the affected axolotl, the left nostril appeared slightly deformed dorsally by a subcutaneous mass. Oral examination confirmed the presence of a 1-cm-diameter, irregular, pink intraoral mass adherent to the left side of the rostral portion of the palate **(Figure 1)**.

Evaluation of the oral mass with the axolotl sedated was elected to assess adherence of the mass to the palate and possible penetration into adjacent anatomic structures. The axolotl was sedated with alfaxalone (5 mg of alfaxalone/L [0.02 mg/kg] of oxygenated water in a 2-L immersion bath). To limit pain sensation, 1% tetracaine (5 mg/0.5 mL [4.2 mg/kg]) was administered directly onto the mass and surrounding mucosa 1 minute prior to manipulation of the mass. The evaluation was performed with sterile cotton swabs to prevent trauma and revealed penetration into adjacent tissue via a fistula that appeared to communicate with the left nasal fossa. Minor traction on the buccal portion of the mass resulted in its removal, including a portion extending through the fistula. After mass removal, the presence

Figure 1—Photograph of a 4-year-old sexually intact male axolotl (*Ambystoma mexicanum*) evaluated because of a 2-week history of dysrexia and difficulty swallowing. A 1-cm-diameter, irregular, pink intraoral mass adherent to the left side of the rostral portion of the palate can be seen to the right of the cotton swab.

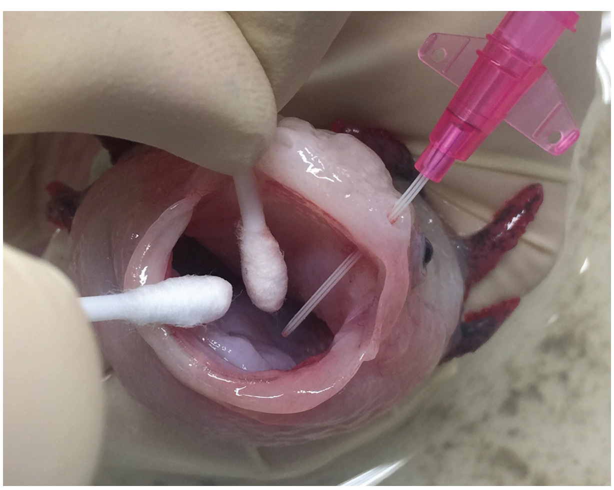

Figure 2—Photograph obtained after excisional biopsy of the oral mass. An oronasal fistula was present, allowing communication with the left nasal fossa, as confirmed with a 20-gauge polypropylene IV catheter with the stylet removed.

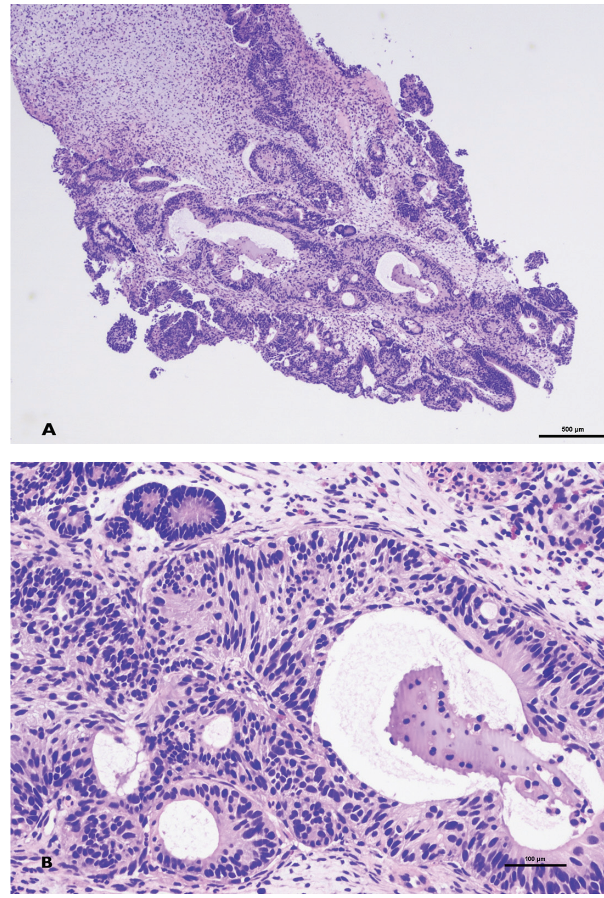

Figure 3—Photomicrographs of a section of the oral mass; the mass was identified as an olfactory neuroblastoma. A—Neoplastic cells are arranged in tubular structures or multilayered rosettes embedded in a loose collagen stroma. The tubules sporadically contain proteinaceous material and cellular debris. H&E stain; bar = 500 μm. B—Neoplastic cells are polygonal to cylindrical with fibrillar eosinophilic cytoplasm and a rounded to elongated nucleus with finely stippled chromatin. H&E stain; bar = 100 μm.

of an oronasal fistula was confirmed with a 20-gauge polypropylene IV catheter with the stylet removed **(Figure 2)**. The mass was placed in neutral-buffered 10% formalin and submitted for histologic examination. Considering the lack of clinical signs postoperatively and the rapid resolution of the dysrexia, no treatment was initiated at that time.

On histologic examination, the mass was composed of small tumor cells forming beams, single-layered tubular structures, and multilayered Flexner-Wintersteiner–like rosettes embedded in a loose collagen stroma **(Figure 3)**. The tubular structures sporadically contained proteinaceous material admixed with cellular debris. The cells were polygonal to cylindrical with moderately abundant, finely fibrillar, eosinophilic cytoplasm and variably distinct cell borders. The nucleus was rounded to elongated with finely stippled chromatin. Anisocytosis and anisokaryosis were mild, and 2 mitoses/hpf (400X) were seen. No healthy tissue was identified. The histologic findings were consistent with an incompletely excised neuroectodermal tumor. Considering the location of the mass, an olfactory neuroblastoma (ONB) was diagnosed.

Given the tissue regenerative capacities of axolotls and the lack of data on this neoplasm in this species, the owners agreed to monitor the progression of the neoplasm before considering adjuvant treatments. Five weeks after mass removal, physical examination revealed persistent erythema, delayed healing of the rostral portion of the palate, and a mild facial deformity associated with a white mass in the nasal cavity **(Figure 4)**. Because the initial excision had been incomplete, the tumor appeared to have recurred, and this type of tumor is known to exhibit malignant behavior in other species, non–con-

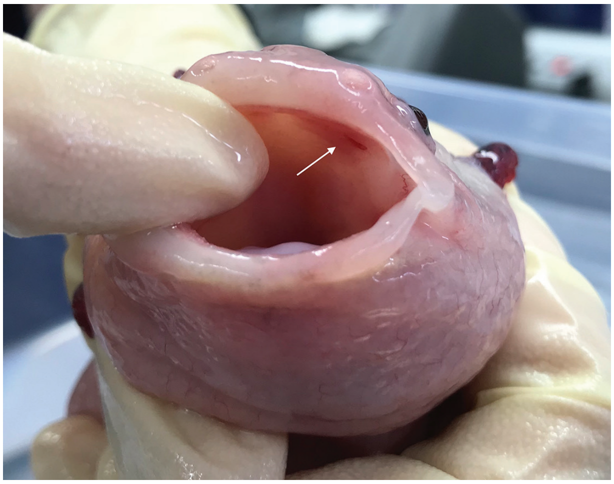

Figure 4—Photograph obtained 5 weeks after excisional biopsy of the oral mass. Persistent erythema and delayed healing of the palate can be seen (arrow).

trast-enhanced full-body CT was performed with a 64-slice CT unit (Brilliance CT 64 channel DS; Philips NV) for staging purposes. To undergo CT, the axolotl was anesthetized by placing it in a bath of fresh water with alfaxalone (5 mg/L [0.02 mg/kg]). Branchial and transcutaneous irrigation with alfaxalone (drop-by-drop administration of 15 mg of alfaxalone diluted in saline [0.9% NaCl] solution [62.8 mg/kg]) was then performed until the axolotl was completely immobile. Contrast-enhanced CT was not performed because of budgetary considerations. No clinically important findings were observed on CT images, possibly because of the lack of spatial resolution for small, superficial lesions.

Given the reported incomplete removal of the mass, persistent erythema, and likely presence of a recurrent infiltrative mass, adjuvant electron beam radiotherapy (Precise; Elekta) was initiated. A more invasive surgical approach had been proposed to the owners, taking into account the regenerative capacities of axolotls. However, for ethical and animal welfare–related reasons, the owners preferred the less disruptive approach of radiotherapy. In contrast to the anesthetic protocol used for CT, during which immobility was the primary concern, radiotherapy required that the animal be sufficiently anesthetized that it would tolerate the weight of a 5-mm tissue-equivalent bolus material (wet gauze) on its head and insertion of a 2-mm-thick lead plate into its mouth for beam-blocking purposes **(Figures 5 and 6)**. Therefore, an anesthesia protocol that consisted of alfaxalone administered by branchial and transcutaneous irrigation and that would allow for rapid reversal following each radiotherapy session by placing the animal in successive baths of fresh water was selected. Data on efficacy of alfaxalone following IM administration have not been published for this species. Therefore, branchial and transcutaneous irrigation was chosen to mitigate the anesthetic risk. Various protocols were used during the course of radiotherapy **(Table 1)**, and doses, time to reach maximal anesthetic effect, degree of im-

Figure 5—Photograph obtained during radiotherapy. A 5-mm tissue-equivalent bolus material (wet gauze) has been placed on the animal's head to optimize surface dose. The treatment couch has been rotated by 90°, and the gantry has been angled so that the electron applicator (6 X 6 cm with blocks at each corner to create a hexagonal shape) is parallel to the skin.

Figure 6—Photograph obtained during radiotherapy. The animal has been positioned in sternal recumbency, and a custom-made 2-mm-thick lead plate has been inserted in the oral cavity to protect the mandible from radiation exposure.

mobility without stimulus, resistance to stimulation consisting of placement of gauze on the animal's head, and resistance to insertion of the metal plate into the mouth were recorded. The general protocol involved

Table 1—Anesthetic protocols used during radiotherapy of an olfactory neuroblastoma in a 4-year-old sexually intact male axolotl (*Ambystoma mexicanum*).

Dose of alfaxalone	Mean (range) TME (min)	Immobility	RGS	Jaw resistance	RMI
30 mg (125.5 mg/kg)	11 (10–12)	+/–	–	–	–
40 mg (167.4 mg/kg)	15.25 (14–17)	+	+/–	–	–
50 mg (209.2 mg/kg)	20.75 (20–22)	+	+	+	+/–
60 mg (251 mg/kg)	29 (26–31)	+	+	+	+

The anesthetic protocol involved mixing 1 mL of alfaxalone (10 mg/mL solution) in 1 mL of saline (0.9% NaCl) solution. This mixture, which contained 10 mg of alfaxalone (41.8 mg/kg), was then instilled by branchial and transcutaneous irrigation for over 1 to 2 minutes. After 2 minutes, immobility was evaluated by checking for a righting reflex. As long as the reflex was still present, an additional 2-mL mixture was prepared and applied in the same way. This process was repeated until immobility and the other desired effects were observed. Effects were scored as present for at least 15 minutes (+), present but not maintained for > 2 minutes (+/−), or absent (−).

RGS = Resistance to gauze stimulation (ie, placement of wet gauze [5-mm tissue-equivalent bolus material] on the animal's head). RMI = Resistance to insertion of a 2-mm-thick lead plate into the animal's mouth for beam-blocking purposes.TME = Time to reach maximal effect.

mixing 1 mL of alfaxalone (10 mg/mL solution) in 1 mL of saline solution. This mixture, which contained 10 mg of alfaxalone (41.8 mg/kg), was then instilled by branchial and transcutaneous irrigation for over 1 to 2 minutes. After 2 minutes, immobility was evaluated by checking for a righting reflex. As long as the reflex was still present, an additional 2-mL mixture was prepared and applied in the same way. This process was repeated until immobility and the other desired effects were observed. In this case, a sufficient level of anesthesia was achieved with 60 mg of alfaxalone (diluted in 12 mL of saline solution [ie, 6 mixtures containing 10 mg of alfaxalone each]). The animal required 15 to 25 minutes to recover. A succession of 3 freshwater baths (16 to 18 °C) was used to facilitate elimination of the alfaxalone. An excitation phase was observed after each anesthetic period, which was attenuated by immediate transfer to a clean water bath. No abnormalities were noticed by the owners following each radiotherapy session.

The elected radiotherapy protocol consisted of 4 weekly fractions of 8 Gy each (6 MeV; 2 Gy/min), for a total dose of 32 Gy. The patient was positioned in sternal recumbency with a custom-made 2-mm-thick lead plate in its oral cavity to protect the mandible from radiation exposure. A 5-mm tissue-equivalent bolus material (wet gauze) was placed on its head to optimize surface dose. The treatment couch was rotated by 90°, and the gantry was angled rostrally so that the electron applicator (6 X 6 cm with blocks at each corner to create a hexagonal shape) was parallel to the skin and the isocenter was centered on the visible mass, covering a margin of approximately 2 cm around the lesion. A 6-MeV beam was used, and the dose was manually calculated and set to the 90% isodose line (5-mm depth into tissue). No acute adverse radiation effects were noted following radiotherapy. The oral erythema completely disappeared after the third session.

One month after completion of radiotherapy, the axolotl showed normal behavior and a good appetite. Non–contrast-enhanced full-body CT was performed by use of the same anesthetic protocol as the initial CT and did not reveal any clinically important findings. No recurrence was observed 2 months after treatment, and the axolotl showed no appreciable clinical abnormalities at home. Fourteen weeks after radiotherapy, the axolotl died secondary to an environmental management failure during the summer that allowed the tank water to overheat. The other 2 axolotls living in the same tank also died.

Necropsy was performed, and the entire head and various organ samples (liver, spleen, heart, lung, testicle, kidney, stomach, small intestine, and large intestine) were submitted for histologic examination. Seven sections of the head, including the brain, and 1 section of each of the other samples were examined. No tumor cells were identified in any of the sections, suggesting complete remission. Necropsy findings were consistent with acute septicemia and edema secondary to heat shock.

Discussion

Although there are similarities in the cellular structures of humans and amphibians, spontaneous tumors are rare in amphibians.[1–3] Spontaneous tumors that have been reported in axolotls include melanoma,[4] epithelioma,[2] neuroepithelioma,[5] ONB,[6] lymphangiosarcoma,[7] mast cell tumor,[8] and teratoma.[9] However, there are few detailed studies regarding these tumors. Furthermore, treatment was attempted only for the axolotl with lymphangiosarcoma,[7] in which the lesion was removed surgically.

Olfactory neuroblastoma, also known as esthesioneuroblastoma, is an uncommon malignant neoplasm of the nasal cavity arising from the olfactory neuroepithelium.[10] The upper respiratory system in adult axolotls includes the external nares, narial ducts, internal nares (choana), buccopharyngeal cavity, glottis, larynx, and trachea.[11] The choana, bounded in part by the vomeropalatinum and located laterally between the 2 rows of functional teeth, has a slot-like appearance.[12] In the case reported here, the buccal mass communicated with the nasal cavity via an oronasal fistula located rostromedially to the internal nares and medially to the rows of teeth. Invasion and adherence of the inner part of the mass to the nasal cavity through the fistula were observed during its removal, indicating that the mass originated from the nasal cavity.

In amphibian medicine, ONB appears to be an underrepresented condition, with only 2 published reports[5,6] of axolotls in which ONB was identified at necropsy. In the report of Shioda et al,[6] the histologic characteristics were similar to those observed in our case. In the other report,[5] the lesion was described as a neuroepithelioma but appeared to have the same histologic characteristics as described by Shioda et al[6] in 2011. No treatment was attempted in either case.

In human medicine, ONB was first described in 1924 and remains a rare condition. It accounts for 3% of all intranasal tumors, with a reported incidence of 4 cases/10 million people.[13] In the veterinary literature, reports of ONBs as spontaneous tumors are infrequent, but a few cases have been described involving dogs, cats, cattle, monkeys, horses, fish, and transgenic mice.[13–22] Olfactory neuroblastoma has also been described in laboratory rats[23] and hamsters[24] in which tumor growth was induced with carcinogens. It is interesting to note that some of these chemicals are found in food (such as aspartame) or cigarette smoke (such as nitrosamines). The authors suggested that these molecules could act as inducers in pet species[23,24]; however, further studies are required.

Owing to their rarity and complex nature, the origin and etiology of ONBs still remain unclear. In 1990, 3 cats infected with feline and murine leukemia type C retrovirus that developed ONB were reported.[25] Similarly, a pathogenic agent has been observed in transgenic mice that developed ONB as a result of activation of endogenous human adenoviruses,[22] and ONB has been observed simultaneously with distemper virus in a dog.[26] However, the role of viruses in the development of ONB needs further exploration. In the case reported here, no etiology was identified.

Computed tomography and MRI with and without contrast are the first-line methods for evaluation of ONBs in human medicine.[10,27–29] A classification system based on extent of the tumor is commonly used in human medicine and associated with survival rate.[10] This system, called the Kadish staging classification, has been used in veterinary medicine, but survival rates have not yet been associated with the various stages.[14] In this system, stage A refers to a tumor confined to the nasal cavity, stage B refers to a tumor involving the nasal cavity in addition to 1 or more paranasal sinuses, and stage C refers to extension of the tumor beyond the nasal cavity and sinuses. According to this system, the tumor described in the present report would be classified as stage C because it extended into the oral cavity.

In veterinary medicine, it has been suggested that a diagnosis of ONB is best achieved with MRI.[30–32] However, a more recent study[30] found that a definitive diagnosis could not be made on the basis of signal intensity or contrast enhancement. Nevertheless, the authors concluded that the presence of a mass in the caudal aspect of the nasal cavity with extension into the neurocranium seemed to be a characteristic highly indicative of ONB.

A definitive diagnosis of ONB is best achieved through histologic and immunohistochemical analysis,[13] and immunohistochemistry helps to differentiate ONB from other intranasal tumors. However, immunohistologic profiles differ from one species to another, with each species having a specific response to the antigen.[13] In the case described here, immunohistochemistry was not performed because of budgetary considerations.

Concerning the management of ONB, the standard in human medicine, based on an extensive narrative review of 3,876 articles related to ONB,[10] is surgical resection followed by radiotherapy. In veterinary medicine, only 5 articles[15,16,32–34] describe management of ONB, including diagnostic methods and survival times. Surgical excision associated with radiotherapy yields the best survival times, with a remaining life span of up to 20 months according to 1 study.[33] As in human medicine, it has been observed that surgery without adjunctive therapy is associated with a high likelihood of metastasis and short survival time.[34] Without surgery or radiotherapy, reported survival times are short, ranging from only 5 to 23 days.[16,32]

In the case described in the present report, the combination of excisional biopsy and radiotherapy led to complete remission, as indicated by postmortem examination. The decision to administer 32 Gy over 4 sessions was driven by results in other species as well as logistic and financial considerations.[10,15]

Analgesia should be considered in axolotls when implementing various treatment options, given that their perception of pain is similar to that of other amphibians.[35,36] In amphibians, it is recommended to use opioids (eg, buprenorphine or butorphanol) and NSAIDs (eg, meloxicam) in conjunction with a balanced anesthetic protocol to ensure patient comfort during and after surgery.[37,38] However, studies[39–41] regarding analgesics in axolotl are lacking. In the case described here, analgesia could have been improved during and after the excisional biopsy by administering other drugs in addition to tetracaine, especially given the lack of analgesic effect of alfaxalone.[39–41]

Considering the tissue regenerative capacity of axolotls, it is fair to question whether radiotherapy would have any effect on them. The only studies evaluating the impact of radiation on axolotls were published before 1960, the most relevant of which considered the reaction of limb regenerates to x-irradiation.[42] In 90% of the animals irradiated with a single dose of 4,000 rad (40 Gy) and in all of those irradiated with a single dose of 6,000 rad (60 Gy), reduction of the regenerates was observed during the 45- to 125-day period of evaluation. The skin epithelium reduced gradually, without any formation of open wounds.

In conclusion, findings for the axolotl described in the present report suggest that ONB should be considered in the differential diagnosis of an intraoral palatal mass. A combination of excisional biopsy and radiotherapy was successfully used to treat the ONB in this animal, with no tumor cells seen in postmortem sections examined 3 months after radiotherapy.

References

1. Green DE, Harshbarger JC. Spontaneous neoplasia in amphibian. In: Wright KM, Whitaker BR, eds. *Amphibian Medicine and Captive Husbandry*. Krieger Publishing Co; 2001:335–400.
2. Balls M. Spontaneous neoplasms in Amphibia: a review and descriptions of six new cases. *Cancer Res.* 1962;22:1142–1154.
3. Duryee WR, Long ME, Taylor HC Jr, et al. Human and amphibian neoplasms compared. *Science.* 1960;131(3396):276–280.
4. Sheremetieva EA. Spontaneous melanoma in regenerating tails of axolotls. *J Exp Zool.* 1965;158:101–122.
5. Brunst VV, Roque AL. Tumors in amphibians. I. Histology of a neuroepithelioma in *Siredon mexicanum*. *J Natl Cancer Inst.* 1967;38(2):193–204.
6. Shioda C, Uchida K, Nakayama H. Pathological features of olfactory neuroblastoma in an axolotl (*Ambystoma mexicanum*). *J Vet Med Sci.* 2011;73(8):1109–1111.
7. Menger B, Vogt PM, Jacobsen ID, et al. Resection of a large intra-abdominal tumor in the Mexican axolotl: a case report. *Vet Surg.* 2010;39(2):232–233.
8. Harshbarger JC, Chang SC, DeLanney LE, Rose FL, Green DE. Cutaneous mastocytomas in the neotenic caudate amphibians *Ambystoma mexicanum* (axolotl) and *Ambystoma tigrinum* (tiger salamander). *J Cancer Res Clin Oncol.* 1999;125(3-4):187–192.
9. Brunst VV, Roque AL. A spontaneous teratoma in an axolotl (*Siredon mexicanum*). *Cancer Res.* 1969;29(1):223–229.
10. Fiani B, Quadri SA, Cathel A, et al. Esthesioneuroblastoma: a comprehensive review of diagnosis, management, and current treatment options. *World Neurosurg.* 2019;126:194–211.
11. Zug GR, Vitt LJ, Caldwell JP. Anatomy of amphibians and reptiles. In: Zug GR, Vitt LJ, Caldwell JP, eds. *Herpetology: an Introductory Biology of Amphibians and Reptiles.* Academic Press; 2001:33–76.
12. Clemen G, Greven H. Organisation of the palate in a spontaneously transforming Mexican axolotl (*Ambystoma mexicanum*): a case report. *Vertebr Zool.* 2015;65(3):351–356.
13. Lubojemska A, Borejko M, Czapiewski P, Dziadzuiszko R, Biernat W. Of mice and men: olfactory neuroblastoma among animals and humans. *Vet Comp Oncol.* 2014;14(3):e70–e82. doi:10.1111/vco.12102
14. Brosinski K, Janik D, Polkinghorne A, Von Bomhard W, Schmahl W. Olfactory neuroblastoma in dogs and cats—a histological and immunohistochemical analysis. *J Comp Pathol*. 2012;146(2-3):152–159.
15. Ueno H, Kobayashi Y, Yamada K. Olfactory esthesioneuroblastoma treated with orthovoltage radiotherapy in a dog. *Aust Vet J*. 2007;85(7):271–275.
16. Parker VJ, Morrison JA, Yaeger MJ. Olfactory neuroblastoma in a cat. *J Feline Med Surg*. 2010;12(11):867–871.
17. Anderson BC, Cordy DR. Olfactory neuroblastoma in a heifer. *Vet Pathol*. 1981;18(4):536–540.
18. Döpke C, Gröne A, von Borstel M, von Oppen T, Boéve MH, Baumgärtner W. Metastatic esthesioneuroblastoma in a horse. *J Comp Pathol*. 2005;1329(1-2):218–222.
19. Yamate J, Izawa T, Ogata K, et al. Olfactory neuroblastoma in a horse. *J Vet Med Sci.* 2006;68(5):495–498.
20. Vigliano FA, Marcaccini AJ, Sarradell J, Bermúdez R, Quiroga MI. First description of an olfactory neuroblastoma in goldfish *Carassius auratus*: a case report. *Dis Aquat Organ.* 2011;96(1):61–68.
21. Torikata C, Mukai M, Kageyama K. Spontaneous olfactory neuroepithelioma in a domestic medaka (*Oryzias latipes*). *Cancer Res.* 1989;49(11):2994–2998.
22. Koike K, Jay G, Hartley JW, Schrenzel MD, Higgins RJ, Hinrichs SH. Activation of retrovirus in transgenic mice: association with development of olfactory neuroblastoma. *J Virol.* 1990;64(8):3988–3991.
23. Takagi M, Shiraiwa K, Kusuoka O, Tamura K. A case of olfactory neuroblastoma induced in a rat by n-nitrosobis(2-hydroxypropul)amine. *J Toxicol Pathol.* 2010;23(2):111–114.
24. Herrold KM. Induction of olfactory neuroepithelial tumors in Syrian hamsters by diethylnitrosamine. 1964;17:114–121.
25. Schrenzel MD, Higgins RJ, Hinrichs SH, Smith MO, Torten M. Type C retroviral expression in spontaneous feline olfactory neuroblastomas. *Acta Neuropathol*. 1990;80(5):547–553.
26. Candini D, Biasato I, Dell'Armelina Rocha PR, Grego E, Capucchio MT, Vercelli C. How behavioral changes can indicate serious cerebral pathology: a case report of concomitant olfactory neuroblastoma and distemper virus encephalitis in a Swiss Shepherd Dog. *Vet Sci.* 2017;4(3):42–46.
27. Pickuth D, Heywang-Kobrunner SH, Spielmann RP. Computed tomography and magnetic resonance imaging features of olfactory neuroblastoma: an analysis of 22 cases. *Clin Otolaryngol Allied Sci*. 1999;24(5):457–461.
28. Yousem DM, Oguz KK, Li C. Imaging of the olfactory system. *Semin Ultrasound CT MR*. 2001;22(6):456–472.
29. Kairemo KJ, Jekunen AP, Kestila MS, Ramsay HA. Imaging of olfactory neuroblastoma—an analysis of 17 cases. *Auris Nasus Larynx*. 1998;25(2):173–179.
30. Söffler C, Hartmann A, Gorgas D, et al. Magnetic resonance imaging features of esthesioneuroblastoma in three dogs and one cat. *Tierarzt Prax Ausg K Kleintiere Heimtiere*. 2017;44(5):333–340.
31. Troxel MT, Vite CH, Massicotte C, et al. Magnetic resonance imaging features of feline intracranial neoplasia: retrospective analysis of 46 cats. *J Vet Intern Med.* 2004;18(2):176–189.
32. Kitagawa M, Okada M, Yamamura H, Kanayama K, Sakai T. Diagnosis of olfactory neuroblastoma in a dog by magnetic resonance imaging. *Vet Rec.* 2006;159(9):288–289.
33. Gumpel E, Moore AS, Simpson DJ, Hoffmann KL, Taylor DP. Long-term control of olfactory neuroblastoma in a dog treated with surgery and radiation therapy. *Aust Vet J.* 2017;95(7):227–231.
34. Siudak K, Klingler M, Schmidt MJ, Herden C. Metastasizing esthesioneuroblastoma in a dog. *Vet Pathol.* 2015;52(4):692–695.
35. Llaniguez JT, Szczepaniak MA, Rickman BH, Gelovani JG, Hish GA, Cotroneo TM. Quantitative and qualitative behavioral measurements to assess pain in axolotls (*Ambystoma mexicanum*). *J Am Assoc Lab Anim Sci.* 2020;59(2):186–196.
36. Stevens CW. Relative analgesic potency of mu, delta and kappa opioids after spinal administration in amphibians. *J Pharmacol Exp Ther.* 1996;276(2):440–448.
37. Coble DJ, Taylor DK, Mook DM. Analgesic effects of meloxicam, morphine sulfate, flunixin meglumine, and xylazine hydrochloride in African-clawed frogs *(Xenopus laevis)*. *J Am Assoc Lab Anim Sci.* 2011;50(3):355–360.
38. Koeller CA. Comparison of buprenorphine and butorphanol analgesia in the eastern red-spotted newt (*Notophthalmus viridescens*). *J Am Assoc Lab Anim Sci*. 2009;48(2):171–175.
39. Čermáková E, Oliveri M, Ceplecha V, Knotek Z. Anesthesia with intramuscular administration of alfaxalone in Spanish ribbed newt (*Pleurodeles waltl*). *J Exot Pet Med.* 2020;33:23–26.
40. Huang CL. Dual actions of tetracaine on intramembrane charge in amphibian striated muscle. *J Physiol*. 1997;501(pt 3):589–606.
41. Chatigny F, Kamunde C, Creighton CM, Stevens ED. Uses and doses of local anesthetics in fish, amphibians, and reptiles. *J Am Assoc Lab Anim Sci*. 2017;56(3):244–253.
42. Brunst VV. Reaction of limb regenerates of adult axolotl (*Siredon mexicanum*) to X-irradiation. *Radiat Res*. 1960;12(6):642–656.