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NOTE

Pathology

Primary pharyngeal alveolar rhabdomyosarcoma in an adolescent Japanese black heifer

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ABSTRACT. Alveolar rhabdomyosarcoma (ARMS) is a rare mesenchymal tumor with differentiation toward the skeletal muscle. Although several cases of canine ARMS have been reported in veterinary medicine, only one case of abdominal ARMS has been reported in a cow. A 13-month-old, Japanese black heifer was referred for pus-like nasal discharge. On autopsy, an 11 × 7 × 4.5-cm pedunculated mass closed to the left palatine tonsillar sinus that occupied the laryngopharynx. Histopathological and immunohistochemical analyses indicated that the tumor was a typical ARMS. To the best of our knowledge, this has been the first case of primary pharyngeal ARMS in a Japanese black heifer, which is rare among cows. Nonetheless, its characteristics, including site, age and subtype, are identical to those among humans and dogs.

KEY WORDS: alveolar rhabdomyosarcoma, Japanese black heifer, pharynx

Alveolar rhabdomyosarcoma (ARMS), one of the four categories of human and animal rhabdomyosarcomas (RMSs), is a rare mesenchymal tumor exhibiting variable differentiation to the skeletal muscle [6, 9, 11, 27]. Despite being more prevalent in the extremities, human ARMS can occur anywhere in the soft tissues with or without skeletal muscle, such as the head and neck region including the oropharynx [11, 27]. Although unknown in animals, human ARMS, unlike other RMSs, has a high rate of specific chromosomal translocations and expresses the derivative fusion genes and related products [10, 11, 27]. Recent studies have suggested that this tumor originates from mesenchymal stem cells and not skeletal muscle based on several researches related with the fusion genes [8].

Although several cases of canine ARMS have been reported in veterinary medicine, only one case of ARMS in a cow has been reported [17]. We herein report a bovine case of ARMS located adjacent to the palatine tonsil.

A 13-month-old Japanese black heifer was referred to the Kagoshima University Veterinary Teaching Hospital for pus-like nasal discharge. Physical examination revealed that food debris, not pus, had been discharged from both nostrils. Oral examination could not be performed given the lack of appropriate instruments. The clinician suspected oropharyngeal disorder for which anti-inflammatory and other drugs were provided for approximately 5 months. However, the symptoms did not improve. She was eventually euthanized due to poor prognosis and submitted for autopsy.

On gross inspection, an 11 × 7 × 4.5-cm pedunculated mass was found close to the left palatine tonsillar sinus and occupied the laryngopharynx (Fig. 1A). The surface of the mass was dark to pale red in color with yellow-green exudates and expansive ulceration. The cut surface was fleshy and mildly swollen, edematous, and white to red in color due to mild hemorrhage (Fig. 1B). The mass had a solid texture, was sectioned by fibrous septa and included a focal abscess. Collected tissues were fixed in 10% buffered formalin, routinely processed, and embedded in paraffin wax. Sections (3-μm-thick) were then stained with hematoxylin and eosin and phosphotungstic acid hematoxylin (PTAH) and used for labeled-polymer immunohistochemistry (Histofine Simple Stain MAX PO; Nichirei Biosciences, Tokyo, Japan). The types, suppliers, primary antibody dilution rates, and antigen-retrieval conditions are summarized in Table 1.

Histopathological examination revealed that the tumor was mainly composed of small neoplastic cells in alveolar and solid patterns separated by the fine fibrovascular septa (Fig. 1C). The alveoli contained the degenerated neoplastic cells at the center. The solid structures comprised densely packed small cells, occasionally showed central accumulation of eosinophilic material and erythrocytes, and displayed cystic appearance. This neoplasm was also mixed with a small number of eosinophilic plump cells, and elongate and round multinuclear giant cells (MGCs). The major neoplastic cells had a scant round to polygonal cytoplasm and

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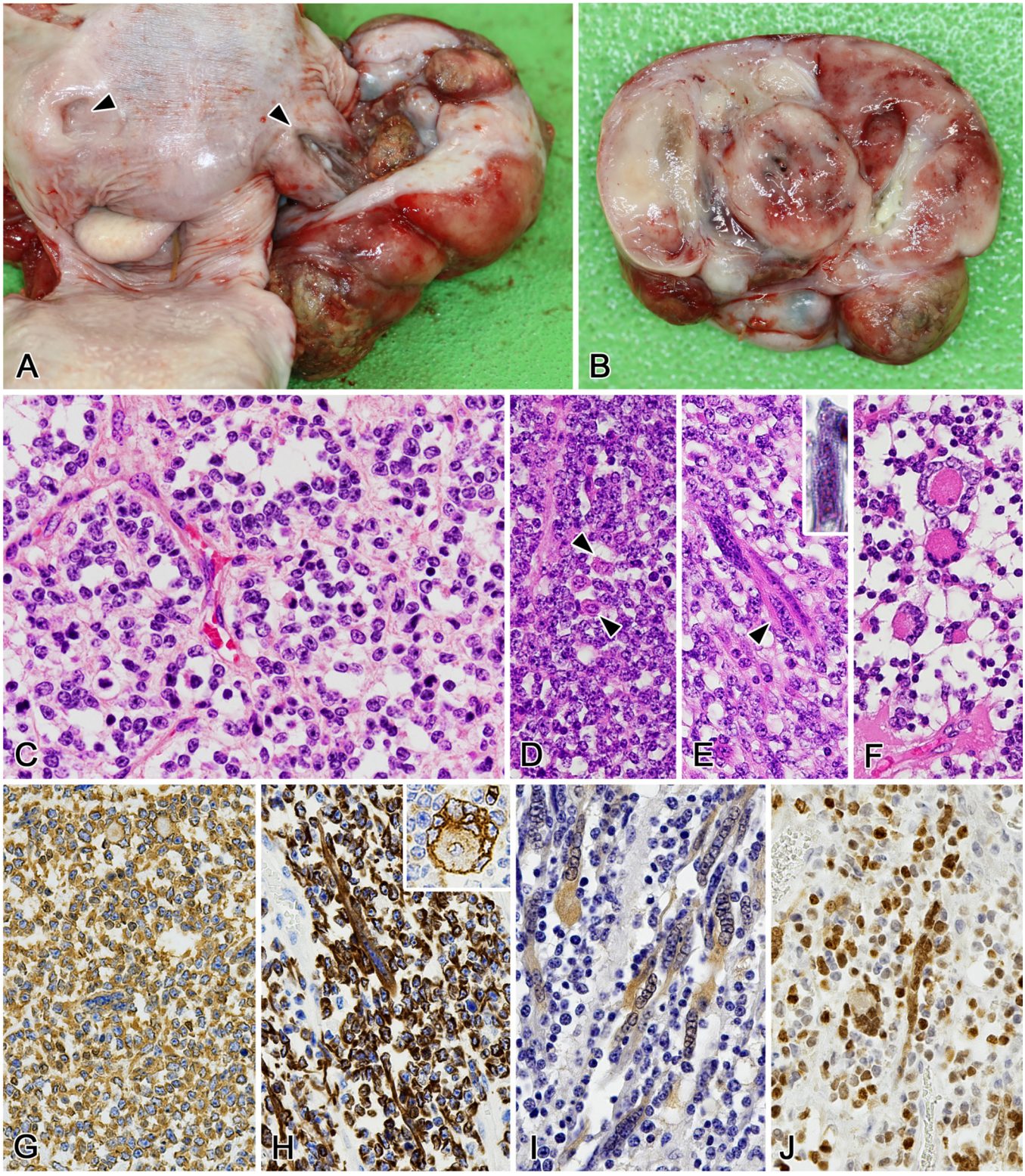

Fig. 1. Pharyngeal alveolar rhabdomyosarcoma in an adolescent Japanese black heifer. (A) A pedunculated mass with expansive ulceration closes to the left palatine tonsillar sinus. Arrowheads indicate the palatine tonsillar sinuses. (B) The cross-section of the mass. The cut surface shows fleshy and edematous appearance, with mildly swelling. The color is white to red owing to mild hemorrhage. (C) Histological findings. The small neoplastic cells proliferate and form alveolar structures, which include degenerated neoplastic cells at the center, and is separated by the fine fibrovascular stroma. Hematoxylin and eosin (HE). (D) The proliferation of the neoplastic cells composes of small cells (the major type) and the intermingled myoblastic cells (arrowheads). HE. (E) Elongated cells include eosinophilic long cytoplasm with cross-striations and nuclear alignment (arrowhead), showing a myotube-like appearance. HE. Inset: Evident cross-striations of the elongated neoplastic cell on phosphotungstic acid hematoxylin staining. (F) Round multinuclear giant cells have multiple nuclei arranged at the periphery of the cytoplasm (i.e., wreath-like cells). HE. (G–J) Reactivity of the neoplastic cells for immunohistochemistry. (G) Vimentin. (H) Desmin. Inset: the wreath-like cell also exhibits reactivity. (I) Myoglobin. (J) Myogenin.

Table 1. Primary antibodies, antigen retrieval conditions, and immunohistochemistry results

Antibody	Clone	Source	Dilution	Antigen retrieval	Reactivity of the neoplastic cells			
					Small cells	Myoblastic cells	Myotube-like MGCs	Wreath-like MGCs
Vimentin	3B4	Dako, Glostrup, Denmark	1:200	121°C, 15 min, in CB	+	+	+	+
Desmin	D33	Dako	(Prediluted)	121°C, 15 min, in CB	+/–	+	+	+/–
Myoglobin	(Polyclonal)	Dako	1:1,000	–	–	+	+	–
Myogenin	F5D	Origene, Rockville, MD, USA	1:200	121°C, 15 min, in CB	+/–	+	+	+

MGCs: multinuclear giant cells, CB: citrate buffer (pH 6.0), +: positive, –: negative, +/–: both positive cells and negative cells were observed.

a round to ovoid nucleus with obvious nucleoli and clumped chromatin, not indicating apparent differentiation (Fig. 1D), whereas the eosinophilic plump cells exhibited a rhabdomyoblastic appearance (Fig. 1D). The elongated MGCs showed nuclear alignment and occasional cross-striations resembling a myotube (Fig. 1E), which could be clearly observed through PTAH staining (Fig. 1E, inset). The round MGCs containing multiple nuclei arranged in the periphery exhibited the so-called "wreath-like" appearance (Fig. 1F). The mitotic count was 40 cells/10 high power fields (400×, 0.237 mm^2). Immunohistochemistry results are summarized in Table 1. The small cells were consistently positive for anti-vimentin antibody and negative for anti-myoglobin antibody while been occasionally negative for anti-desmin and myogenin antibodies. Moreover, the myoblastic and myotube-like MGCs were positive for all antibodies used (Fig. 1G–J), whereas the wreath-like MGCs exhibited positivity for anti-myogenin and vimentin antibodies and absolutely negativity for anti-myoglobin antibody. Although many of the wreath-like MGCs reacted with anti-desmin antibody (Fig. 1H, inset), some non-reactive MGCs were present albeit scattered.

The histological and immunohistochemical aspects of the current neoplasm were identical to those for typical ARMS: poorly differentiated small cells proliferating in the alveoli and solid patterns separated by fibrovascular septa; mixture of myoblastic cells and MGCs, especially wreath-like MGCs; and immunohistochemical reactivity to muscular markers. Typically ARMS mainly consists of small primitive cells showing uniform round nuclear and cytoplasmic shape [9, 11, 27]. Although few differentiated myoblastic cells may be found, cross-striations in such cells are rarely detected [9, 27]. Wreath-like MGCs, a profound feature of this type tumor, can appear and are important clue for differential diagnosis in human cases [27]. The aggregation of the neoplastic cells is supported by fibrovascular septa, whereas neoplastic cells at the center of the nests dissociate and "float" unlike peripheral cells that adhere to the surrounding stroma [9, 11, 27]. Thus, a characteristic histological structure, namely "alveolar" appearance, is formed. Solid variant of ARMS in human and dogs consists of densely packed nests or sheets without fibrovascular septa and "entirely" lack an alveolar pattern [11, 19, 21, 27]. In the present case, although solid structures were occasionally formed, classical alveolar patterns were also contained. Thus, the present case was diagnosed as typical ARMS but not solid variant. Only one report had previously described bovine ARMS forming in the abdominal cavity [17].

Several immunohistochemical markers, especially myogenic antibodies, have been used for the definitive diagnosis of RMSs. Given that marker expression in the RMS cells reflects the degree of differentiation similar to non-neoplastic myocytes and that a number of markers also express non-skeletal muscle lineage, pathologists have to use a set of antibodies for diagnosis. Myogenin, one of the myogenic transcription factors, has been associated with the differentiation from myoblast to myotube, cell proliferation under embryonal development and regeneration [6, 12]. Considering that myogenin exhibited high specificity and sensibility in human RMSs, particularly for histologically undifferentiated neoplastic cells [7, 26], it has been recognized as a reliable diagnostic marker. Anti-myogenin antibody was used in two bovine RMSs and reacted with neoplastic cells [14, 24]. Since the anti-myogenin antibody also exhibited reactivity for all types of neoplastic cells of this case, the antibody can be useful in diagnosing RMSs in cows. Although vimentin is expressed in the early stages of differentiation and is conspicuously downregulated in the final maturation stage [2], many other mesenchymal cells contain vimentin. Desmin is expressed in the early stages of muscular differentiation and remains in mature myofibers [2, 18]. This intermediate filament is also expressed in smooth muscle, cardiac muscle and myofibroblasts [2, 6, 18]. Both vimentin and desmin have been used to indicate poorly differentiated cells of RMSs. In particular, desmin, which is widely expressed in various types of RMS neoplastic cells, had considerable diagnostic utility [22, 23]. Myoglobin, a small molecular weight heme protein expressed in myoblasts or more mature cells, is present in skeletal and cardiac muscle [4, 5, 20]. Thus, immature cells display no or infrequent myoglobin expression. Indeed, small undifferentiated cells in RMSs did not react to or only slightly reacted with anti-myoglobin serum in humans [4]. The histological features and immunohistochemical patterns of neoplastic cells studied herein were almost identical those above.

ARMSs in humans tend to occur among adolescents to young adults [16, 27]. Moreover, among the eight canine ARMS cases, six affected dogs were under 2 years old [6, 21]. Similar to humans and dogs, this case is considered the typical age of RMS although the only reported case of bovine ARMS occurred in a 7-year-old cow [17].

The most common site for human ARMS has been the extremities, followed by the head and neck region, including the nasal/paranasal region, larynx and oropharynx [17]. However, five of the eight canine ARMSs occurred in the upper gingiva and

Table 2. Histological type, breed, age and site of bovine rhabdomyosarcomas reported in the literature

Type	Breed	Age	Site	Reference
Alveolar	Holstein	7 y	Peritoneum and pleura (multiple)	Matsui *et al*. [17]
Embryonal	Holstein	(calf)	Subctis of the head	Ulrich *et al*. [24]
Embryonal	Holstein	63 m	Lung and LNs of the body cavity	Kachi *et al*. [14]
Embryonal	Holstein	2 y	Pleura	Jimma *et al*. [13]
ND	Holstein	7 m	Pleura and omentum (multiple)	Kajiwara *et al*. [15]
Pleomorphic	Holstein	2 y	Heart	Taylor *et al*. [23]
Pleomorphic	Holstein	5 y	Skeletal muscle of the chest	Aoyagi *et al*. [1]
Pleomorphic	Crossbred	15 y	Skeletal muscle around the femur	Bisby *et al*. [3]

ND: Not descripted, y: year, m: month, LN: lymph node.

maxillofacial region [6, 21], suggesting that ARMS among dogs tends to affect the head. Although eight bovine RMS cases have been reported (Table 2), only one bovine ARMS case has been published [17]. Moreover, among the eight bovine RMS cases, only one occurred in the head, but not the oropharynx, regardless of category [25]. The most common sites for bovine cases include the body cavity and body cavity organs without skeletal muscle [13–15, 17], although two cases of skeletal muscle origin and one case of myocardial origin have been reported [1, 3, 24]. Thus, the current case can be considered rare among cows in terms of the involved site and RMS subtype but share identical characteristics with human and canine cases.

Seven of the eight reported bovine cases involved Holstein cows with varying ages (Table 2). The high incidence of RMSs among Holstein breeds may be due to their high breeding numbers, considering the popularity of the breed, or unknown genetic predispositions.

To the best of our knowledge, this has been the first case report on primary pharyngeal ARMS in a Japanese black heifer.

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