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Rhabdomyosarcoma in a racing pigeon (*Columba livia*)

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An adult racing pigeon (*Columba livia*) was presented with a subcutaneous mass on the ventral aspect of the right wing. A fine-needle aspirate and radiographic study of the mass were suggestive of highly invasive sarcomatous neoplasm. Euthanasia was decided because of the poor prognosis. Necropsy confirmed the highly invasive nature of the neoplasm, which also occupied a large portion of the right breast. There also was extensive osteolysis of the sternum with neoplastic invasion of the left breast and the coelomic cavity. Histopathology revealed a highly cellular, poorly demarcated, unencapsulated invasive sarcoma. Immunohistochemistry was positive for muscle actin, and myoglobin, weakly positive for vimentin, and negative for desmin, neuron-specific enolase and S-100 protein, suggesting a diagnosis of undifferentiated rhabdomyosarcoma.

Introduction

Benign or malignant neoplasms originating from striated or smooth muscle are observed occasionally in captive and free-ranging birds. Rhabdomyosarcomas have often been described as irregular, elevated, lobulated, relatively firm subcutaneous swellings of the wing or shoulder that limit the use of the wing (Latimer, 1994). Reported sites of origin also include the eye (Dukes & Pettit, 1983), tongue and eyelid (Latimer, 1994), dorsum (Raphael & Nguyen, 1980), and head (Duncan & Fitzgerald, 1997), but they are rarely observed in birds (Reece, 1992; Schmidt, 1997) and have not been described in pigeons. Microscopically, these neoplasms are composed of a pleomorphic population of fusiform to elongated cells. Anisokaryosis may be prominent with plump oval-to-elongated nuclei, and some elongated or 'strap cells' will retain cross striations typical of skeletal muscle cells (Latimer, 1994). Confirmation of the diagnosis requires the use of electron microscopy or immunohistochemistry. In the first case, the most useful diagnostic feature is the presence of myofilaments. In the latter case, antibodies that have proven useful include those against vimentin, desmin, actin, myoglobin, myosin and titin in several species of mammals (Cooper & Valentine, 2002); and muscle actin, vimentin and desmin in several species of birds (Frazier et al., 1993; Duncan & Fitzgerald, 1997; Hafner et al., 1998; Ijzer et al., 2002).

In the present paper we describe a primary rhabdomyosarcoma in the ventral musculature of the right wing. To our knowledge this is the first case of the description of this tumour in a pigeon with confirmation by immunohistochemical techniques.

Case History

An adult racing pigeon (*Columba livia*) was presented to the veterinary practice with a firm, non-ulcerated, lobulated, subcutaneous mass on the ventral aspect of the right wing that encompassed the distal humerus and proximal ulna and radius. The size and localization of the mass precluded flight, although the general condition of the bird was good. A fine-needle aspirate of the mass was obtained and cytological examination

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revealed numerous exfoliated cells arranged singly or in clusters. Cells were round to oval, and were variable in size with an irregularly basophilic cytoplasm that was finely vacuolated in some cells. Nuclei were often oval, but some were very large and irregularly shaped, with one or two prominent nucleoli. Multinucleated cells and mitoses were also observed. There was also a moderate amount of a pink material between the cells (Figure 1). The cytological diagnosis was a malignant sarcomatous neoplasm of stromal cells. Radiographic examination under general anaesthesia with isofluorane was performed to determine the extent of the neoplasm. There was a large soft tissue mass, increased opacity in the right humerus and ulna, and a lytic lesion involving the distal humerus near the joint. A lateral radiographic view revealed increased opacity in the sternum and pectoral muscle area. Because of the diagnosis of a sarcomatous malignant neoplasm, euthanasia was selected. At necropsy, the neoplastic growth was multilobular with multiple pinkish grey spherical nodules of different sizes (0.5 to 3 cm.). The growth was associated with osteolysis of the humerus and ulna, and invaded a large part of the right breast. The tumour invaded through the sternum at multiple sites with extension into the left breast and into the precordial thoracic cavity. The internal organs did not exhibit apparent lesions.

**Materials and Methods**

The tumoural mass and all major organs and tissues were fixed in 4% neutral buffered formalin, processed routinely, sectioned at 4 μm and sections were stained with haematoxylin and eosin, and phosphotungstic acid haematoxylin. Additional immunohistochemical stains were carried out on the tumoural tissue. The primary antibodies, antigen retrieval methods, and incubation conditions used are described in Table 1. Biotinylated goat anti-mouse or goat anti-rabbit antibodies (as appropriate) were used as secondary antibodies, followed by the avidin–biotin complex immunoperoxidase procedure (Naish et al., 1989). Diaminobenzidine with 0.5% H₂O₂ in imidazol buffer (pH 7.2, 0.1 M) was used as chromogen. Replicated sections of tumoural tissue were immunostained using normal rabbit serum (S-100 protein, desmin, myoglobin) and normal mouse serum (NSE, vimentin, muscle actin) instead of the primary antiseraum, as negative controls. Finally, sections of leg muscles of the same bird were used as positive controls.

**Results**

Histologically, sections of the mass were highly cellular, unencapsulated, and invasive. The tumour was composed of anaplastic fusiform to elongated cells arranged in irregular sheets that lacked apparent stroma, and showed focal areas of necrosis. Neoplastic cells had abundant eosinophilic cytoplasm but lacked cross striations, as was demonstrated on replicated sections stained with phosphotungstic acid haematoxylin. Anisokaryosis was prominent, with plump oval-to-elongated nuclei, coarsely clumped to vacuolated chromatin and prominent nucleoli. Mitotic figures were common, some of them irregular in shape. Cells containing multiple nuclei were also common (Figure 2).

Neoplastic cells were immunopositive for muscle actin and myoglobin, but negative for desmin. Immunostaining for myoglobin was homogeneous and intense, while immunostaining for actin was intense only in scattered neoplastic cells (Figure 3). Staining for vimentin, an intermediate filament that labels a variety of mesenchymal cells, was homogeneous, albeit much lighter than for myoglobin. Staining for the remaining cell markers (desmin, S-100, NSE) was absent in the tumour cells.

In the sections of leg muscle, used as positive controls, striated muscle cells were vimentin negative, and desmin, muscle actin and myoglobin positive. As expected, peripheral nerves (Schwann cells) were S-100 and NSE positive; and non-muscle mesenchymal cells and vascular endothelial cells were vimentin positive.

**Table 1.** Primary antibodies, antigen retrieval methods, and incubation conditions used in the immunohistochemical tests performed

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Host species</th>
<th>Target species</th>
<th>Source</th>
<th>Antigen retrieval</th>
<th>Dilution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Desmin</td>
<td>Rabbit</td>
<td>Chicken</td>
<td>DakoCytomation(^a)</td>
<td>–</td>
<td>1:50</td>
</tr>
<tr>
<td>Muscle actin</td>
<td>Mouse</td>
<td>Human</td>
<td>DakoCytomation(^a)</td>
<td>Saponin</td>
<td>1:100</td>
</tr>
<tr>
<td>Myoglobin</td>
<td>Rabbit</td>
<td>Human</td>
<td>DakoCytomation(^a)</td>
<td>Saponin</td>
<td>1:350</td>
</tr>
<tr>
<td>Neuron-specific enolase (NSE)</td>
<td>Mouse</td>
<td>Human</td>
<td>DakoCytomation(^a)</td>
<td>–</td>
<td>1:800</td>
</tr>
<tr>
<td>S-100 protein</td>
<td>Rabbit</td>
<td>Cow</td>
<td>DakoCytomation(^a)</td>
<td>–</td>
<td>1:400</td>
</tr>
<tr>
<td>Vimentin</td>
<td>Mouse</td>
<td>Pig</td>
<td>DakoCytomation(^a)</td>
<td>–</td>
<td>1:50</td>
</tr>
</tbody>
</table>

\(^a\) DakoCytomation Denmark A/S, Glostrup, Denmark.
Discussion

Immunohistochemistry is now the preferred diagnostic technique for confirmation of the diagnosis of rhabdomyosarcoma and has been utilized in a great number of recent studies (Cooper & Valentine, 2002). Histologically, the undifferentiated sarcomatous characteristics of this tumour could also have been consistent with fibrosarcoma or schwannoma. In fact fibrosarcoma and schwannoma are usually also positive for vimentin and negative for desmin, as were the cells of this tumour. Nonetheless, positive staining for myoglobin and muscle actin, and lack of immunostaining for S-100 suggest the diagnosis of rhabdomyosarcoma (True, 1990).

Of the immunohistochemical markers used on this neoplasm, the pattern of positive staining most clearly matched the staining characteristics of rhabdomyomas and rhabdomyosarcomas, since neoplastic cells were muscle actin and myoglobin positive and S-100 and NSE negative (True, 1990; Cooper & Valentine, 2002). However, the negative staining for desmin and positive staining for vimentin presented a possible discrepancy between our results and the expected staining pattern of rhabdomyosarcomas. This apparent inconsistency can be resolved since antigens are expressed differently depending on the degree of differentiation of the rhabdomyocytes, probably reflecting the sequence of expression of antigens in the developing rhabdomyocytes. Vimentin is expressed early in the rhabdomyocytic development but is later lost, while desmin and actin are also expressed early but persist (Carter et al., 1990; Wijnaendts et al., 1994). In fact, in domestic mammals, vimentin is often expressed in interstitial cells and is usually absent from well-differentiated rhabdomyosarcomas, but may be expressed in poorly differentiated rhabdomyoblasts (Cooper & Valentine, 2002). Also, desmin has been undetectable in some pleomorphic rhabdomyosarcomas in humans (Furlong et al., 2001). Thus, the weak homogeneous positive staining for vimentin, and absence of staining for desmin in the neoplastic cells in this case can be interpreted as a sign of poor differentiation.

The irregular staining for actin could be due to the heterogeneity of the neoplastic cells, with positive cells being those most well differentiated, whereas cells negative for actin would be more anaplastic (Cooper & Valentine, 2002). The intense homogeneous staining for myoglobin observed in this case is difficult to account for under this scenario, as this protein is expressed late during differentiation (Carter et al., 1990).

We can conclude that the histologic features and the immunocytochemical profile of the tumour cells were consistent with a diagnosis of a poorly differentiated pleomorphic rhabdomyosarcoma.

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References

Rhabdomyosarcome chez un pigeon voyageur (Columbia livia)

Un pigeon voyageur adulte (Columbia livia) soumis à consultation présentait une masse sous-cutanée à l’aile droite en position ventrale. Une étude radiographique et une aspiration à l’aide d’une aiguille fine de cette masse ont suggéré qu’il s’agissait d’un néoplasme sarcomateux très fortement invasif. L’euthanasie de l’animal a été décidée du fait du pronostic pessimiste. L’autopsie a confirmé la nature très invasive de ce néoplasme qui occupait une zone importante de la partie droite du brèchet. Il y avait également une importante ostéolyse du sternum avec une invasion néoplasticque du côté gauche du brèchet et de la cavité céphalique. L’examen histopathologique a révélé un sarcome invasif, non encapsulé, peu délimité et fortement cellulaire. L’immunohistochimie a été positive pour l’actine du muscle et la myoglobine, faiblement positive pour la vimentine et négative pour la desmine, l’enolase spécifique du neurone et la protéine S-100, suggérant un diagnostic de rhabdomyosarcome indifférencié.

RESUMEN

Rhabdiosarcoma en una paloma (Columbia livia)

Se presentó una paloma (Columbia livia) adulta con una masa subcutánea en la parte ventral del ala derecha. Una aspiración con aguja fina y un estudio radiográfico de la masa sugrieron una neoplasia sarcomatosa altamente invasiva. Se decidió eutanasiar al animal debido al grave pronóstico. La necropsia confirmó la naturaleza invasiva de la neoplasia, que ocupaba una proporción amplia del pectoral derecho. También se observó una osteólisis extensa del esternón con invasión neoplásica del pectoral izquierdo y de la cavidad céfalo. La histopatología reveló un sarcoma invasivo no encapsulado, densamente celular y mal delmarcado. La immunohistoquímica fue positiva para la actina y mioglobina muscular, débilmente positiva para la vimentina y negativa para la desmina, enolasa específica de neuronas y la proteína S-100, lo que sugiere un diagnóstico de rhabdiosarcoma no diferenciado.