NOTE *Pathology*

Primary Ischial Osteosarcoma Occupying the Pelvic Cavity in a Japanese Black Cow

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(Received 7 October 2013/Accepted 16 January 2014/Published online in J-STAGE 31 January 2014)

ABSTRACT. A 10-year-old Japanese Black cow presented with a swelling of the right femur, and a hard, large mass occupied the pelvic cavity. The mass strongly adhered to the visceral surface of the ischium and had posteriorly invaded among the right femoral muscles. Histologically, the mass was composed of neoplastic osteoblasts and exhibited osteoid and immature trabecular bone production. In the region where the mass adhered to the ischium, neoplastic cells were continuously proliferating into the medullary cavity. Tumor emboli were observed in the small vessels of the femoral muscles and lungs. Based on these findings, the mass was diagnosed as an osteosarcoma and considered to have arisen from the ischium.

KEY WORDS: cattle, ischium, osteosarcoma.

doi: 10.1292/jvms.13-0491; *J. Vet. Med. Sci.* 76(6): 891–894, 2014

Osteosarcoma (OS) is a primary malignant bone tumor and is histologically characterized by the production of osteoid and/or immature bone tissue by neoplastic osteoblasts [11, 12]. OS is the most common bone tumor in dogs, cats and humans and is considered to be one of the most malignant and aggressive tumors as it is associated with a high frequency of pulmonary metastasis. In dogs, primary OS occur more commonly in the appendicular than the axial skeleton. Most OS develop in the metaphysis, and forelimbs are affected twice as often as hindlimbs. In the axial skeleton, approximately 50% of cases occur in the head, e.g., in the mandible or maxilla, and the remaining 50% occur in the vertebral column, ribs and pelvis. Although OS are rare in domestic animals other than dogs and cats, a few cases have been reported in cattle, sheep and horses, in which the OS predominantly developed in the head [12]. In cattle, several cases of OS have been reported, including some that originated in the axial skeleton, e.g., in the maxilla [6, 8], mandible [5] or nasal cavity [7, 14] and others that originated in the appendicular skeleton, e.g., the scapula [10] or metacarpal bone [3]. In bovine species, OS have never been reported to occur in the pelvic bones. In this paper, we report a case of primary OS in the ischium of a Japanese Black cow, in which pulmonary metastasis developed.

A ten-year-old Japanese Black cow presented with a swelling of the right femur. The animal delivered 7 times before this presentation. Based on the absence of an abnormal gait or sores on the swollen femur, a tentative diagnosis of an intramuscular abscess was made, and the cow was treated with antibiotics. However, the swelling persisted despite continuous antibiotic treatment. The cow became emaciated and astatic, and developed an enophthalmos. A hard, large mass was palpated in the pelvic cavity during a rectal examination, but the animal showed no dyschezia or dysuria. Serum biochemistry abnormalities, including increases in the animal's alkaline phosphatase (497 U/*l*; reference range, 27–107 IU/*l*), creatine phosphokinase (11020 U/*l*; reference range, 105–409 IU/*l*) and lactate dehydrogenase (4490 U/*l*; reference range, 697–1445 IU/*l*) [2] levels, were observed. The cow was euthanized due to its poor prognosis.

At necropsy, the cow weighed 375 kg. The intrapelvic mass, which measured about 22 × 20 × 20 cm in size, occupied the large part of the cavity, and the rectum, uterus and urethra had been pushed in the dorsal direction by the mass (Fig. 1). The cut surfaces of the mass revealed white, solid tissue with nests of ossification and extensive necrosis. The intrapelvic mass firmly adhered to the visceral surface of the right ischial table, and the boundary between the mass and the visceral surface of the right ischium was focally continuous near the ischial symphysis (Fig. 2). The mass had grown posteriorly along the surface of the right ischial table and ischial arch and had invaded between the right femoral muscles. Also, neoplastic growth progressed forward along the external surface of the right ischial table via the right ischial arch (Fig. 2). The right sciatic nerve was compressed by the neoplastic mass in the femur. Some femoral muscles, such as the adductor and semimembranosus muscles, were also compressed by the mass. Tumor emboli were observed in the right external pudendal vein and small caliber vessels in the femoral muscles. The muscles around the left hip joints, including the biceps femoris, gracilis, sartorius and adductor magnus muscles, had degenerated. In the lungs, a solid red nodule, which measured 10 mm in diameter, and tumor emboli, up to 10 mm in diameter, were found.

The tissues collected during the necropsy were fixed in 10% phosphate-buffered formalin, embedded in paraffin and sectioned at 4 μm thickness. After fixation, the tissues,

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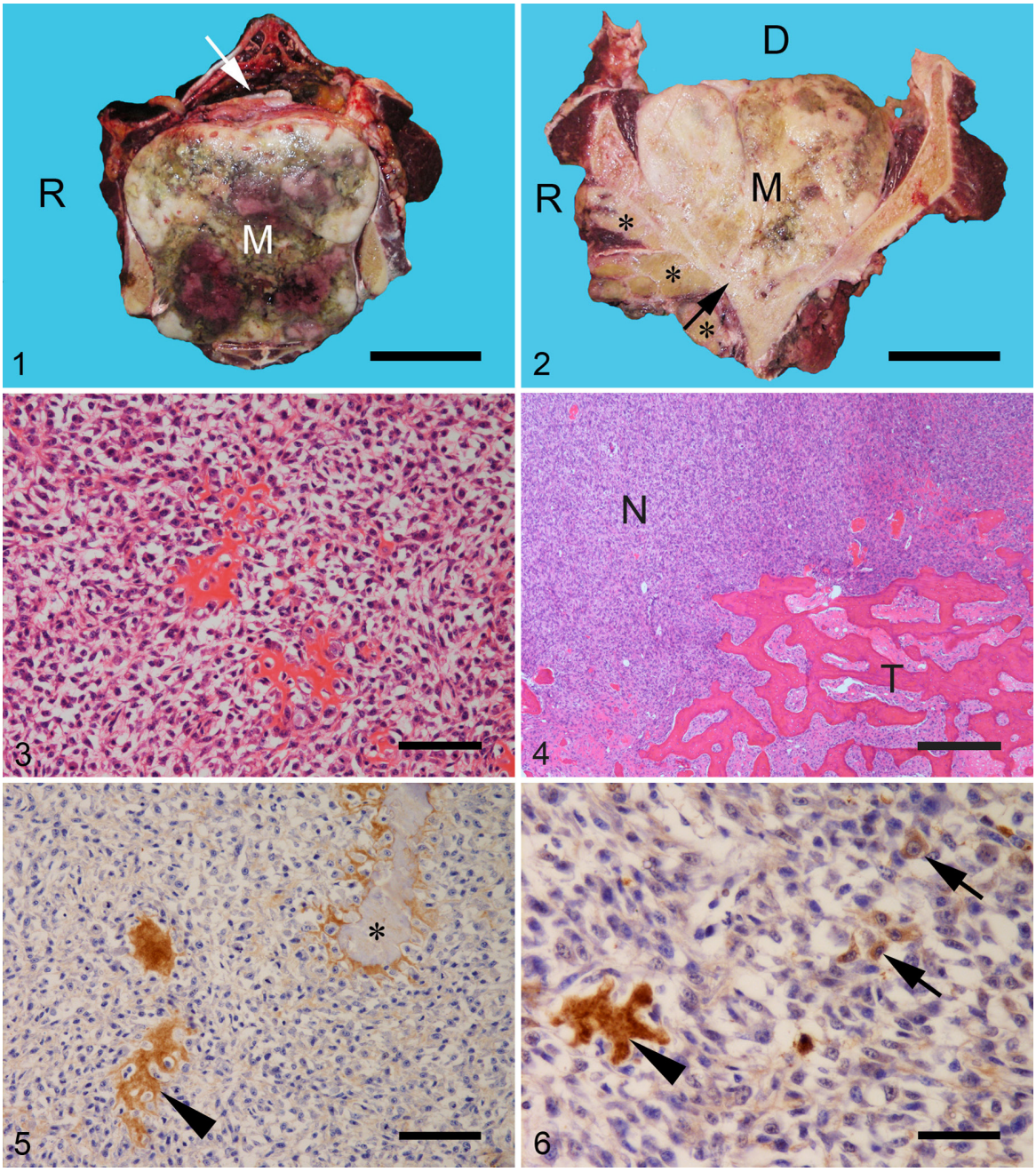

Fig. 1. Intrapelvic mass (M). The mass occupied the large part of the pelvic cavity, and the rectum, uterus and urethra (arrow) had been pushed in the dorsal direction by the mass. The cut surfaces of the mass revealed white, solid tissue with nests of ossification and extensive necrosis. R: right side. Bar=10 cm.

Fig. 2. Transverse section taken from the caudal edge of the obturator foramen showing a continuous transition between the intrapelvic mass (M) and the visceral surface of right ischial table (arrow). Several neoplastic nodules were present outside the right ischial table (asterisks), which extended forward from the posterior part of the intrapelvic mass via the right ischeal arch (not shown in this figure). R: right side, D: dorsal side. Bar=10 cm.

Fig. 3. Intrapelvic mass. The neoplastic osteoblasts had proliferated to form sheets and exhibited multifocal osteoid production. HE. Bar=100 μm.

Fig. 4. The proliferating neoplastic cells had invaded the right ischial plate, resulting in the loss of cortical and trabecular bone. HE. Bar=500 μm, N: neoplastic tissue, T: trabecular bone.

Fig. 5. Intrapelvic mass. Osteoid (arrowhead) and osseous matrix around the calcified core (asterisk) show positive immunoreaction to osteocalcin. Immunohistochemistry for bovine osteocalcin counterstained with hematoxylin. Bar=100 μm.

Fig. 6. Intrapelvic mass. A few osteoblastic tumor cells (arrows) and the osteoid (arrowhead) show positive immunoreaction to osteonectin. Immunohistochemistry for mouse osteonectin counterstained with hematoxylin. Bar=50 μm.

including the osseous matrix, were decalcified using 10% formic acid. The sections were stained with hematoxylin and eosin. Histologically, the intrapelvic mass was composed of densely packed proliferating neoplastic osteoblasts and exhibited the multifocal production of osteoid with/without calcification and immature trabecular bones (Fig. 3). The morphology and arrangement of the neoplastic cells varied regionally. The osteoblastic tumor cells had plump to spindle-shaped cytoplasm and round to ovoid nuclei with one or two nucleoli and had proliferated to form sheets. The fibroblastic tumor cells had spindle-shaped cytoplasm and ovoid to elongated nuclei and had formed fascicles. In some regions, the neoplastic cells showed more anaplastic features, including a highly pleomorphic nucleus with distinct nucleoli and scant cytoplasm that was interconnected with reticular cell processes. The average number of mitotic figures was 3.9 in 10 high-power fields (× 400). Extensive multifocal necrosis was also detected. In the region where the mass adhered to the visceral surface of the right ischium, neoplastic cells had proliferated into the medullary cavity, resulting in the loss of cortical bone, and extensive periosteal and endosteal reactive bone formation were present around the focus (Fig. 4). The intrapelvic mass was not in communication with other bones. The tumor embolus in the external pudendal vein had fused to the intima, and neoplastic growth had invaded the media. In addition to the tumor emboli detected during the gross examination, many microemboli were observed in the surrounding muscles, and the neoplastic cells had broken through the vascular wall and proliferated invasively in the muscle tissue. In the lungs, tumor emboli were observed in the peribronchiolar arteries, and multifocal neoplastic growth was observed in the parenchyma. The neoplastic cells were similar to the anaplastic cells observed in the intrapelvic mass. Metastases were not detected in the lymph nodes, including mandibular, superficial cervical, axillary, superficial inguinal, popliteal, medial and lateral retropharyngeal, tracheobronchial, hepatic, renal and medial and lateral iliac lymph nodes. Immunohistochemistry was performed by the avidin-biotin-peroxidase complex method (Vectastain Elite ABC Kit; Vector Laboratories, Burlingame, CA, U.S.A.). Primary antibodies we used were a mouse monoclonal antibody for bovine osteocalcin (clone OCG3; Abcam, Cambridge, U.K.; diluted 1:200) and a rabbit polyclonal antibody for mouse osteonectin (LSL, Tokyo, Japan; diluted 1:1,600). Antigen retrieval for osteocalcin was performed with pepsin in 0.2 N HCl at 37°C for 30 min. Secondary antibodies we used were biotinated anti-mouse and anti-rabbit IgG (H+L) (Vector Laboratories) for osteocalcin and osteonectin, respectively. Visualization was accomplished using 3,3′-diamino-benzidine. The osteoid and osseous matrix produced by neoplastic cells reacted with osteocalcin and osteonectin, and approximately 8% of osteoblastic tumor cells also reacted with osteonectin (counted in a total of 1,500 cells in 5 high-power fields), but the fibroblastic and anaplastic tumor cells were negative (Figs. 5 and 6).

From its histological and immunohistochemical findings, the intrapelvic mass in the present cow was diagnosed as an osteosarcoma that had metastasized to the lungs. OS can present with a variety of morphologies and neoplastic cell arrangements and are divided into six histological subtypes in domestic animals: the poorly differentiated, osteoblastic, chondroblastic, fibroblastic, telangiectatic and giant cell types [11, 12]. The histology of the present case was consistent with a mixture of the poorly differentiated, osteoblastic and fibroblastic subtypes.

Based on the fact that the tumor was found to be in communication with the right ischium during the gross and histological examinations, the ischium was considered to be the tumor's site of origin. In domestic animals other than dogs and cats, OS occur more commonly in the axial than the appendicular skeleton, in contrast to the situation in dogs, cats and humans [12]. In cattle, all of the reported cases of OS involving the axial skeleton originated in the head [5–8, 14]. To the best of our knowledge, this is the first case of OS to occur in the pelvic bones in cattle.

OS are primary malignant bone tumors that arise associated with bone, but those which are not associated with bone, i.e. extraskeletal osteosarcomas, occasionally develop [12]. According to the primary location of the malignant transformation, bone-associated OS can be divided into two categories: central and peripheral [11, 12]. Central OS originate in the medullary cavity and show extremely aggressive behavior, including a high rate of hematogenous pulmonary metastasis. Peripheral OS occur on the external surfaces of bones and include parosteal OS, which grow slowly outward and display minimal malignant features, and periosteal OS, which are more undifferentiated than parosteal OS. Central OS tend to grow more aggressively than periosteal OS, but it is often difficult to differentiate between these 2 types. Extraskeletal OS arise in soft tissues without primary bone lesion [12]. In our case, the tumor mass developed closely associated with the ischium, but the destruction of the preexisting bone was not so extensive. Based on these considerations, the tumor in the present case might have been a peripheral OS of the ischium, but a precise diagnosis could not be made. None of the reported cases of bovine OS involving the axial skeleton have exhibited metastasis [5–8, 14]. In this case, the tumor mass enlarged expansively and also invaded the vascular system, resulting in pulmonary metastasis.

In dogs, OS that develop in the appendicular skeleton show more malignant biological behavior than those that occur in the axial skeleton [12]. OS in the pelvic bones are also rare in dogs, although a case of iliac OS involving multiple metastases has been reported [9]. However, no study has compared the behavior of OS affecting the pelvic bones with those involving other sites. Our case of ischial OS showed malignant behavior, and the accumulation of more bovine cases will be necessary to determine the correlations between the biological behavior of the neoplasm and neoplastic cell morphology or the tumor's site of origin.

There are several mass-forming lesions that commonly affect the pelvic cavity of bovine species including enlarged lymph nodes due to enzootic leukemia, granulosa cell tumors of the ovary and uterine tumors [1, 13]. In enzootic bovine leukemia, the superficial lymph nodes enlarge. Large lesions in the ovary or uterus can be detected by rectal and/

or ultrasound examinations. As for femur swelling, an intermuscular abscess might be the most likely diagnosis [4]. Although the present case of intrapelvic OS is considered to be extremely rare, we should take account of such lesions during differential diagnosis.

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