

Vaginal clear cell carcinoma in a Japanese Black cow

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ABSTRACT. During artificial insemination of an 18-year-old female Japanese Black cow, a mass that was of a hen's egg size was found in the vagina. On necropsy, the firm mass, measuring approximately $3.5 \times 3.5 \times 3.0$ cm, was located at the superior region of the vagina. The cut surface of the mass was gray-white in color with occasional necrotic or hemorrhagic areas. Histologically, the mass was composed of tumor cells arranged in solid nests of various sizes with an occasional tubular structure separated by a delicate fibrovascular stroma. The tumor cells had a hypochromatic nucleus and abundant, faintly eosinophilic cytoplasm. The tumor cells contained diastase-sensitive periodic acid-Schiff positive granules. Immunohistochemically, tumor cells were positive for cytokeratin AE1/AE3, CAM5.2 and carcinoembryonic antigen, but not for vimentin, p63, estrogen receptor- α , progesterone receptor, α -smooth muscle actin, neuron-specific enolase, S-100 protein and chromogranin A. On the basis of these findings, the tumor was diagnosed as a clear cell carcinoma of the vagina.

KEY WORDS: clear cell carcinoma, Japanese Black cow, tumor, vagina

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Primary tumors arising from the vagina are frequent in women [7]. Six types of glandular tumors of the vagina have been described: clear cell adenocarcinoma, endometrioid adenocarcinoma, mucinous adenocarcinoma, mesonephric adenocarcinoma, Müllerian papilloma and adenoma, according to the Health Organization (WHO) classification of tumors in human [1]. On the other hand, vaginal tumors are extremely uncommon in domestic animals. Vaginal epithelial tumors, such as papillomas, fibropapillomas and squamous cell carcinomas, are listed in the WHO classification of domestic animals [5]. In cattle, primary vaginal tumors, such as adenocarcinoma, leiomyomas, fibromas, fibropapillomas, fibrosarcomas and leiomyosarcomas, have been previously reported [6, 11, 14]. However, vaginal clear cell carcinoma has not been previously reported in domestic animals.

During artificial insemination, a mass that was of a hen's egg size was found in the vagina of an 18-year-old Japanese Black cow. Except for this mass, no abnormalities were observed on physical examination. Complete blood count and routine serum biochemical examinations were not performed. Two months after the discovery of the mass, the cow was euthanized at the owner's request.

On necropsy, the firm mass, measuring approximately $3.5 \times 3.5 \times 3.0$ cm, was located at the superior region of the vagina (Fig. 1). The cut surface of the mass was gray-white in color with occasional necrotic or hemorrhagic areas (Fig. 1,

Inset). The vaginal mucosal surface covering the mass was ulcerated and hemorrhagic. Other organs, including the uterus and ovaries, were macroscopically normal.

The mass was fixed in 10% neutral-buffered formalin and embedded in paraffin. Sections ($4 \mu\text{m}$) were stained with hematoxylin and eosin (HE), periodic acid-Schiff (PAS), mucicarmine, alcian blue and Fontana–Masson's stains. Serial sections were subjected to immunohistochemistry (IHC) using a streptavidin-biotin complex method with primary mouse antibodies specific for cytokeratin (CK) (AE1/AE3, 1:200, Dako, Denmark A/S, Glostrup, Denmark), low molecular weight CK (CAM5.2, prediluted, Becton Dickinson, Franklin Lakes, NJ, U.S.A.), estrogen receptor- α (ER- α , 1:50, Dako), p63 (1:200, NeoMarkers Inc., Fremont, CA, U.S.A.), vimentin (1:100, Dako), α -smooth muscle actin (SMA, 1:400, Dako), neuron-specific enolase (NSE, 1:200, Dako), progesterone receptor (PgR, 1:50, abcam, Cambridge, U.K.) and Ki-67 (1:100, Dako), and primary rabbit antibodies specific for S-100 protein (1:500, Dako), chromogranin A (1:200, Dako) and carcinoembryonic antigen (CEA, 1:300, Dako). The primary antibodies used were validated by noting a positive reaction with their corresponding normal tissues and a negative reaction on replacement with normal mouse or rabbit immunoglobulins.

Histologically, the mass was composed of tumor cells arranged in solid nests of various sizes with occasional tubular structures separated by a delicate fibrovascular stroma (Fig. 2A). The tumor cells invaded the muscle layer and adjacent mucosa (Fig. 2A). The tumor cells had a large round hypochromatic nucleus containing prominent nucleoli and abundant clear cytoplasm with a faintly eosinophilic tint (Fig. 2B). The frequency of mitotic figures was 0–1 per high-power ($400\times$) field. The tumor cells contained diastase-sensitive PAS-positive granules, indicating glycogen content (Fig. 2B, Inset), whereas they were negative for mucicarmine,

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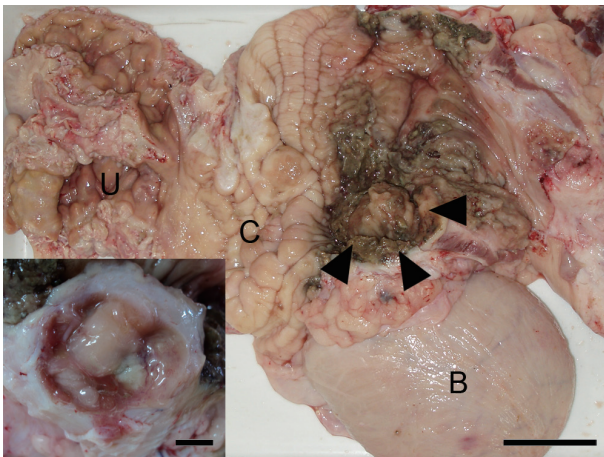


Fig. 1. Vaginal clear cell carcinoma in a cow. A solitary mass (arrowheads) located in the superior region of the vagina. B, urinary bladder; U, uterus; C, cervix. Bar=5 cm. Inset: Cut surface of the mass. Bar=1 cm.

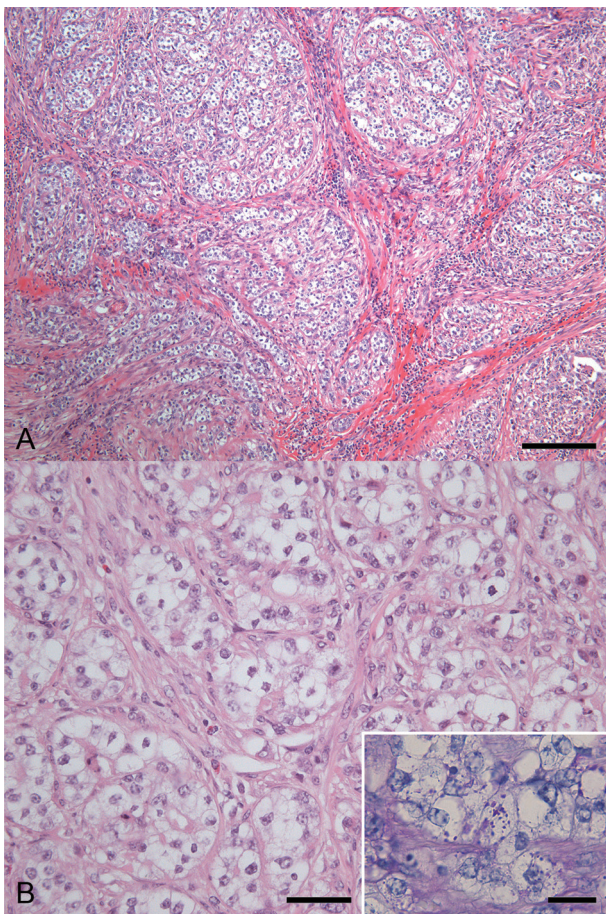


Fig. 2. Vaginal clear cell carcinoma in a cow. A, Tumor cells invaded the muscle layer. HE. Bar=200 μ m. B, Tumor cells arranged in nests separated by a fibrovascular stroma. Tumor cells have round nuclei and abundant clear cytoplasm. HE. Bar=50 μ m. Inset: PAS reaction. Bar=50 μ m.

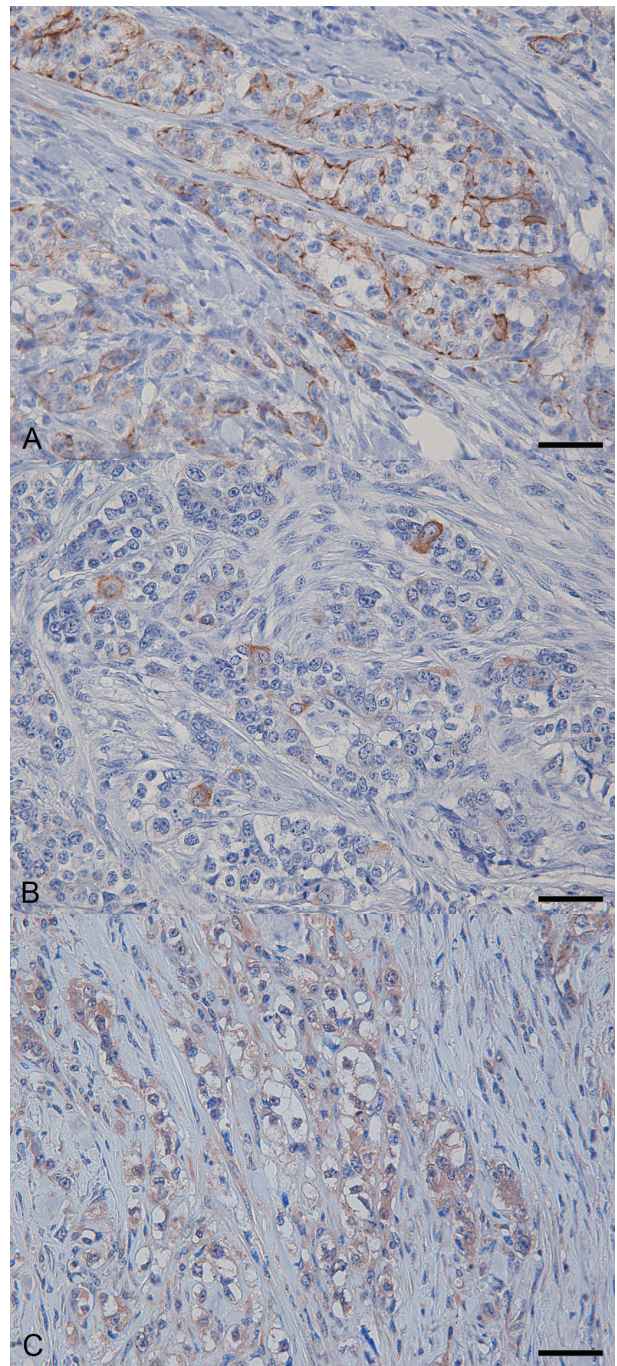


Fig. 3. Vaginal clear cell carcinoma in a cow. A, Expression of CK AE1/AE3 by partial tumor cells. IHC. Bar=50 μ m. B, Expression of CK CAM5.2 by some tumor cells. IHC. Bar=50 μ m. C, Expression of CEA by almost all tumor cells. IHC. Bar=50 μ m.

alcian blue and Fontana–Masson's stains. Lymphocytes and eosinophils infiltrated mildly in stroma. No obvious presence of hobnail cells and/or vascular invasion of tumor cells were observed. Immunohistochemically, tumor cells were positive for CK AE1/AE3 (Fig. 3A), CAM5.2 (Fig. 3B) and CEA (Fig. 3C), but they were negative for vimentin, p63, ER- α , PgR, SMA, NSE, S-100 protein and chromogranin A. The Ki-67 index of tumor cells was low (1.5%).

On the basis of morphological and IHC findings, the tumor was diagnosed as a clear cell carcinoma of the vagina, according to the human WHO classification of tumors of the vagina. In human, clear cell carcinoma occurs frequently in the superior region of the vagina. This tumor shows histologically several growth patterns, such as tubulocystic, solid and/or papillary, and consists of two cell types, clear cell and hobnail cells, often with hyalinizing stroma [1]. Clear cell carcinomas of the reproductive tract express epithelial markers, such as CAM5.2 and CEA, but no hormone receptors, including ER and PgR [13]. In this case, the results of our IHC study are consistent with those performed in humans; however, the proliferation of hobnail cells and hyalinizing stroma was not observed. Clear cell carcinoma can be distinguished from other clear cell tumors, particularly squamous cell carcinoma, neuroendocrine carcinoma and epithelioid leiomyosarcoma, through further IHC analysis. Squamous cell carcinomas contain glycogen granules and retain the expression of p63, which is useful as a diagnostic marker [3]. Neuroendocrine carcinoma is positive for chromogranin A and CK, and negative for p63 [8]. Epithelioid leiomyosarcoma can be ruled out by negative immunostaining for desmin, SMA, ER and PgR [4]. Other vaginal glandular tumors, including endometrioid, mucinous, mesonephric and bartholin adenocarcinomas, do not contain clear cells and intracellular glycogen or mucin [1].

In humans, clear cell carcinoma spread primarily by local invasion and metastases to the pelvic lymph nodes and lungs [1]. Their tumorigenesis is associated with *in utero* exposure to diethylstilbestrol (DES), which leads to precursor cancer lesions, such as vaginal adenosis and endometriosis [2]. DES is a hormone-like product that has been previously used for growth promotion of animals in many countries [9]. However, DES has been banned from beef production, because its carcinogenic and teratogenic properties are a health risk to consumers [7]. Malignant transformation of endometriosis may develop into clear cell carcinoma in individuals without DES exposure [10]. On the other hand, congenital anomalies, such as the persistence of embryonic Müllerian epithelium, can lead to adenosis, which can then transform to clear cell carcinoma [12]. In this case, neither adenosis nor endometriosis was documented in the examined vaginal tissue, although tumor proliferation was observed in the neighboring vaginal mucosa. Therefore, the tumorigenesis of this case remains unclear.

To the best of our knowledge, this is the first report of a clear cell carcinoma of the vagina in a Japanese Black cow.

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