Peritoneal sarcomatoid mesothelioma in a sika deer

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ABSTRACT. A slaughtered 2-year-old female sika deer (*Cervus nippon yesoensis*) had diffusely distributed multinodular lesions on the serosal surface of the peritoneal cavity and several nodules in the pleural cavity. Histologically, they were composed of proliferating spindle-shaped neoplastic cells, arranged in a fascicular fashion. The cells in the invasive foci transitioned from a sarcomatoid to an epithelioid appearance. Immunohistochemically, both the spindle-shaped and epithelioid cells were at least focally positive for pancytokeratin, vimentin, calretinin, α-SMA, and desmin. From these findings, the deer was diagnosed with peritoneal sarcomatoid mesothelioma with metastasis to the pleural cavity. To our knowledge, this is the first reported case of peritoneal mesothelioma in a cervid species and the first case of mesothelioma in a sika deer.

KEY WORDS: *Cervus nippon yesoensis*, neoplasm, peritoneal cavity, sarcomatoid mesothelioma, sika deer

In Japan, as the number of wild sika deer has increased, the numbers of deer that are captured and utilized for venison production have also risen [10]. During fiscal year 2017, about 610,000 deer were captured, and 11% of them were used to produce venison, mostly for human consumption [10]. Information about a broad range of deer diseases is required for safety and hygiene inspections relating to venison production, as is the case for inspections involving domestic food animals, such as pigs and cattle.

Mesothelioma is a neoplastic disorder arising from the mesothelial cells lining the serosal surfaces of the pleura, pericardium, peritoneum, and tunica vaginalis [6, 11]. There has only been one brief report about pleural mesothelioma arising in a deer species (a European spotted fallow deer [*Cervus dama*, now reclassified as *Dama dama*]); i.e., an animal belonging to the family *Cervidae* [4]. In this paper, we present a case of peritoneal mesothelioma with pleural metastasis in a sika deer.

The doe described in the present case was a sika deer (*Cervus nippon yesoensis*), which was captured in the eastern district of Hokkaido, the northern island of Japan, and was farmed in a herd for about a month until it was slaughtered. No clinical or external abnormalities were noted. At slaughter, the doe weighed 62 kg and was estimated to be 2 years old. After opening the abdominal cavity, about 20 *l* of serosanguineous fluid was found in the abdominal cavity. Numerous white, dome-shaped, nodules, which measured a few millimeters to 2 cm in diameter, diffusely covered the serosal surfaces of the gastrointestinal and urogenital tracts, omentum, mesentery, and abdominal wall, and the abdominal side of the diaphragm (Fig. 1a and 1b). In the pleural cavity, at least 10, white, dome-shaped nodules, which measured up to 1 cm in diameter, had formed in the area from the caudal mediastinum to the pleural surface of the diaphragm (Fig. 1c). Some nodules were also detected within the diaphragmatic muscle. A white nodule, which measured 0.5 cm in diameter, was found on the pleura of the left caudal lung lobule. Examinations of cut sections revealed that the nodules consisted of solid white tissue, which was sometimes accompanied by hemorrhagic or yellowish necrotic foci. Liver flukes were detected in the intrahepatic bile duct. The doe was pregnant (crown-rump length of fetus: 32 cm).

Histologically, the nodular lesions in the peritoneal and pleural cavities were composed of neoplastic cells, most of which were proliferating sarcomatoid spindle-shaped cells, arranged in a fascicular fashion, or occasionally in a herringbone or storiform pattern (Fig. 2a). The neoplastic cells had also invaded the underlying fibroadipose connective tissue of the omentum, mesentery, and mediastinum and the muscular tissue of the diaphragm. Irregularly shaped necrotic or hemorrhagic foci were sometimes observed in the neoplastic tissue. The neoplastic cells had eosinophilic spindle-shaped cytoplasm and ovoid to elongated, vesicular nuclei with one or two nucleoli. Mitoses were frequently observed (40 in 10 high-power fields/2.37 mm^2). A few multinucleated giant neoplastic cells were focally scattered. The phrenic and caudal mediastinal lymph nodes had almost been completely replaced by the neoplastic tissue. In the invasive foci, a transition from a sarcomatoid to an epithelioid appearance was observed; i.e., the spindle-shaped neoplastic cells transitioned to macrophage-like, epithelioid cells with moderate to abundant amounts of eosinophilic or occasionally fine granular cytoplasm (Fig. 2b). In the diaphragm, many tumor thrombi were present, and they were predominantly composed of
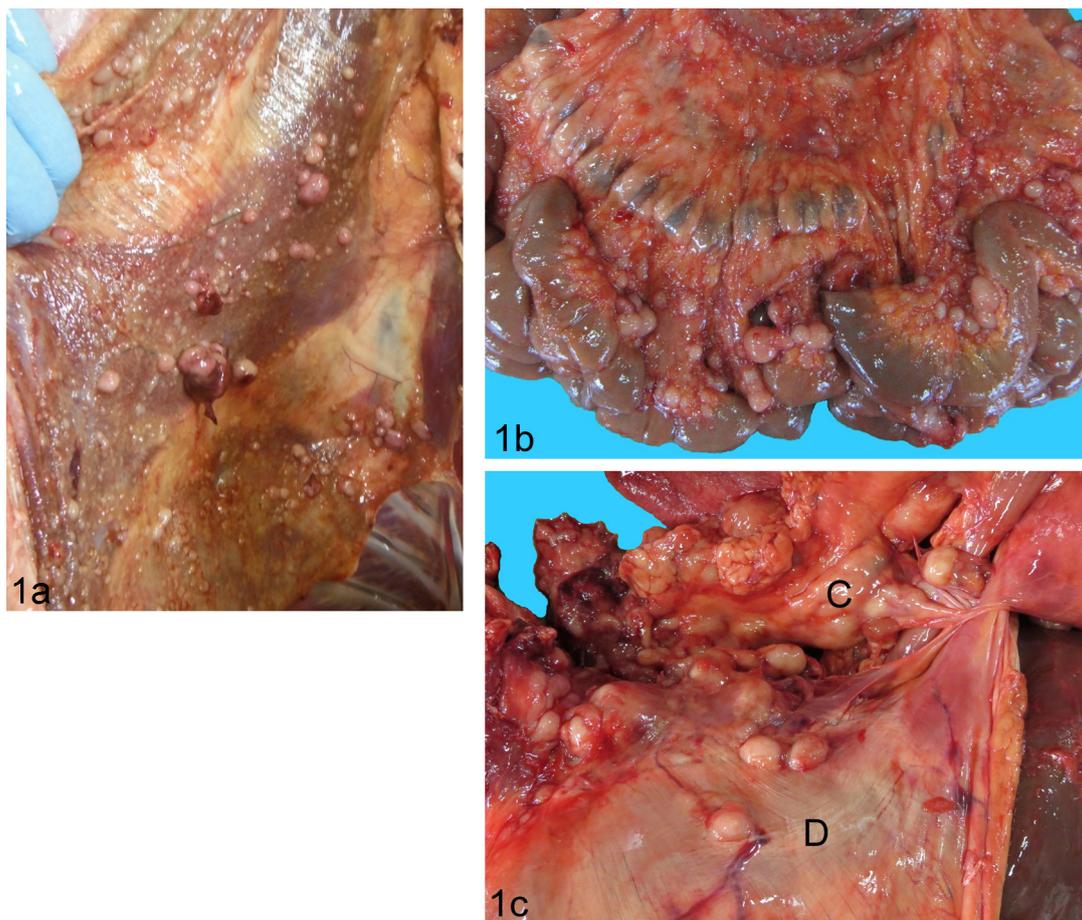
Fig. 1. (a) Numerous, diffusely distributed nodular lesions on the peritoneal surface of the diaphragm in a sika deer. (b) Numerous diffusely distributed nodular lesions on the serosal surfaces of the intestines and mesentery in a sika deer. (c) Nodular lesions on the pleural surface of the diaphragm (D) and an enlarged caudal mediastinal.lymph node (C) in a sika deer.
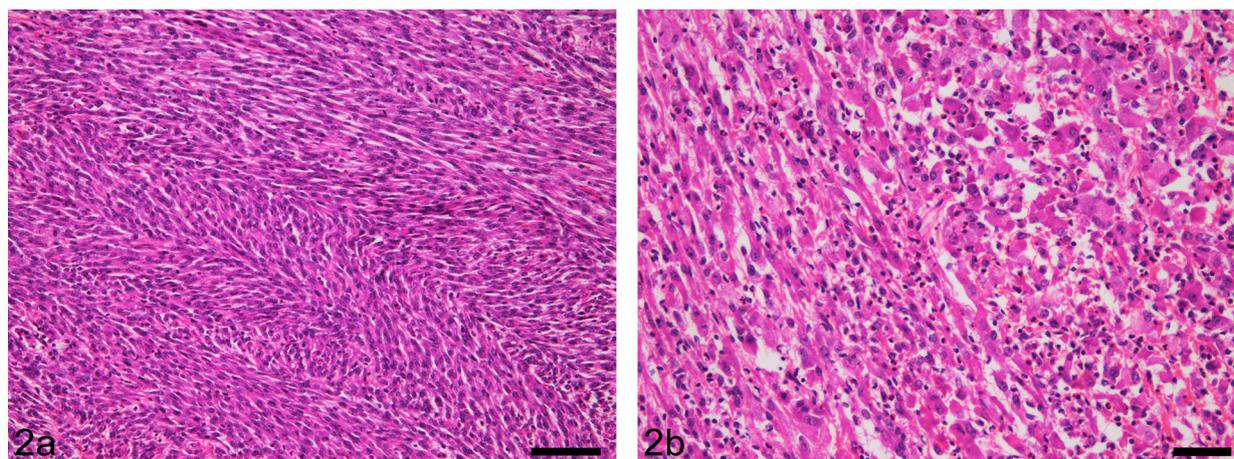
Fig. 2. (a) Proliferating spindle-shaped neoplastic cells arranged in a herringbone pattern in the neoplastic lesion within the diaphragm. Hematoxylin and eosin (HE). Bar=100 μm. (b) Transition from a spindle-shaped to epithelioid neoplastic cell appearance in the neoplastic lesion within the diaphragm. HE. Bar=50 μm.

epithelioid neoplastic cells. The epithelioid neoplastic cells were accompanied by infiltrating neutrophils and lymphocytes, and they occasionally phagocytosed cell debris. The proportion of epithelioid neoplastic tissue was <10% of the total neoplastic mass we examined. Neoplastic cells were absent from the parenchyma of the visceral organs and the fetal tissues.

Table 1. Primary antibodies used for the immunohistochemical examination of a mesothelioma that arose in a sika deer

Antibody	Type (Clone)	Dilution	Antigen retrieval	Source
Pancytokeratin	Mouse, mAb (AE1/AE3)	Prediluted	MW	Nichirei, Tokyo, Japan
Vimentin	Mouse, mAb (V9)	1:100	AC	Dako, Glostrup, Denmark
Calretinin	Rabbit, pAb	1:100	AC	Millipore, Billerica, MA, U.S.A.
Desmin	Mouse, mAb (D9)	1:80	MW	Progen, Heidelberg, Germany
α-SMA	Mouse, mAb (1A4)	1:100	MW	Dako, Glostrup, Denmark

AC, autoclave at 121°C for 15 min in 10 mM citrate buffer, pH6.0; α-SMA, α-smooth muscle actin; mAb, monoclonal antibody; MW, microwave at 500 W for 15 min in 10 mM citrate buffer, pH6.0; pAb, polyclonal antibody.

Table 2. Immunohistochemical results for neoplastic cells from a mesothelioma in a sika deer, the normal mesothelium and positive control tissue from sika deer

Antibody	Mesothelioma		Normal mesothelium of sika deer	Positive control tissue from sika deer
	Spindle-shaped cell	Epithelioid cell		
Pancytokeratin	±	++	++	Skin keratinocytes
Vimentin	+	+	−	Stromal fibroblasts
Calretinin	+	+	+	Cerebellar neurons
Desmin	±	+	−	Striated muscles
α-SMA	+	+	−	Arterial media

++, diffusely positive; +, partially positive; ± , rarely positive; −, negative; α-SMA, α-smooth muscle actin.

Immunohistochemistry was performed using the immunoenzyme polymer method (Histofine Simple Stain MAX-PO, Nichirei, Tokyo, Japan). The primary antibodies we used included mouse monoclonal antibodies against pancytokeratin, vimentin, desmin, α-smooth muscle actin (α-SMA) and a rabbit polyclonal antibody against calretinin (Table 1). We used histological sections of the cerebellum of another sika deer as a positive control for calretinin. The immunohistochemical results, including those for the positive control for each antibody, are provided in Table 2. Most of the spindle-shaped neoplastic cells were negative for pancytokeratin, but epithelioid cells and a few spindle-shaped neoplastic cells were stained for pancytokeratin (Fig. 3a). Of the sarcomatoid neoplastic cells, about 30% of the spindle-shaped cells, especially those with small or slender nuclei, were positively stained for vimentin (Fig. 3b). The epithelioid neoplastic cells were partially positive for vimentin (Fig. 3c). Immunoreactivity for calretinin was observed in both the spindle-shaped and epithelioid neoplastic cells (Fig. 3d). The cerebellar neurons were specifically stained for calretinin (Fig. 3d inset). The spindle-shaped neoplastic cells were frequently positive for α-SMA and rarely positive for desmin, whereas the epithelioid neoplastic cells were sometimes positive for both antigens. Non-neoplastic peritoneal mesothelial cells of another sika deer were immunohistochemically positive for pancytokeratin and calretinin and negative for vimentin, α-SMA, and desmin. Based on the gross, histological, and immunohistochemical findings, a diagnosis of peritoneal sarcomatoid mesothelioma was favored in the present case.

Mesotheliomas are neoplasms that originate from the mesothelial cells lining the serosal surfaces of the pleura, pericardium, peritoneum, and tunica vaginalis, and in terms of their gross appearance they are characterized as diffuse multinodular lesions [6, 11]. In the current sika deer, numerous nodular lesions formed on the serosal surfaces of the peritoneal cavity, and their distribution was consistent with the typical lesion distribution seen in peritoneal mesothelioma.

Histologically, mesotheliomas are classified into epithelioid, sarcomatoid (sarcomatous, fibrous, spindle cell), and biphasic (mixed) types [6, 8, 11]. The epithelioid type is composed of epithelial-like neoplastic cells and can be subdivided into several histological subtypes, such as the papillary, tubular, solid, cystic, and sclerosing subtypes [6, 11]. The sarcomatoid type consists of proliferating plump spindle-shaped neoplastic cells, arranged in bundles, which can form whorls or herringbone patterns [6, 11]. The biphasic type is composed of a mixture of proliferating epithelioid and sarcomatoid cells, although neither cell type predominates [6]. In domestic animals, most reported cases of mesothelioma involved the epithelioid or biphasic type [6]. Single cases of the sarcomatoid type were reported in a dog and a heifer, respectively, and 4 cases have been reported in cats [3, 7, 12, 16]. In the present case, which involved a sika deer, the neoplastic cells were predominantly spindle-shaped, which corresponded to a diagnosis of sarcomatoid mesothelioma. The phagocytic activity displayed by the epithelioid neoplastic cells in the current case might reflect that of normal mesothelial cells [6, 15].

Immunohistochemistry is utilized to definitively diagnose mesotheliomas. Mesothelioma-specific antigens, such as calretinin, cytokeratin 5, Wilms' tumor protein 1, and podoplanin (D2-40), are used in human cases, but immunostaining of these antigens is likely to produce non-specific findings in animal cases [8, 11]. In animals, the dual expression of cytokeratin and vimentin is usually used as a basis for diagnosing mesothelioma [3, 6, 7, 9, 16, 17]. However, cytokeratin immunoreactivity can be focal or absent in human and bovine cases of sarcomatoid mesothelioma [12, 13]. Therefore, the fact that the majority of sarcomatoid cells in the present case did not exhibit immunoreactivity to cytokeratin does not exclude a diagnosis of mesothelioma. On the other hand, the finding of a transition from spindle-shaped to epithelioid cells, which were positively stained for both cytokeratin and
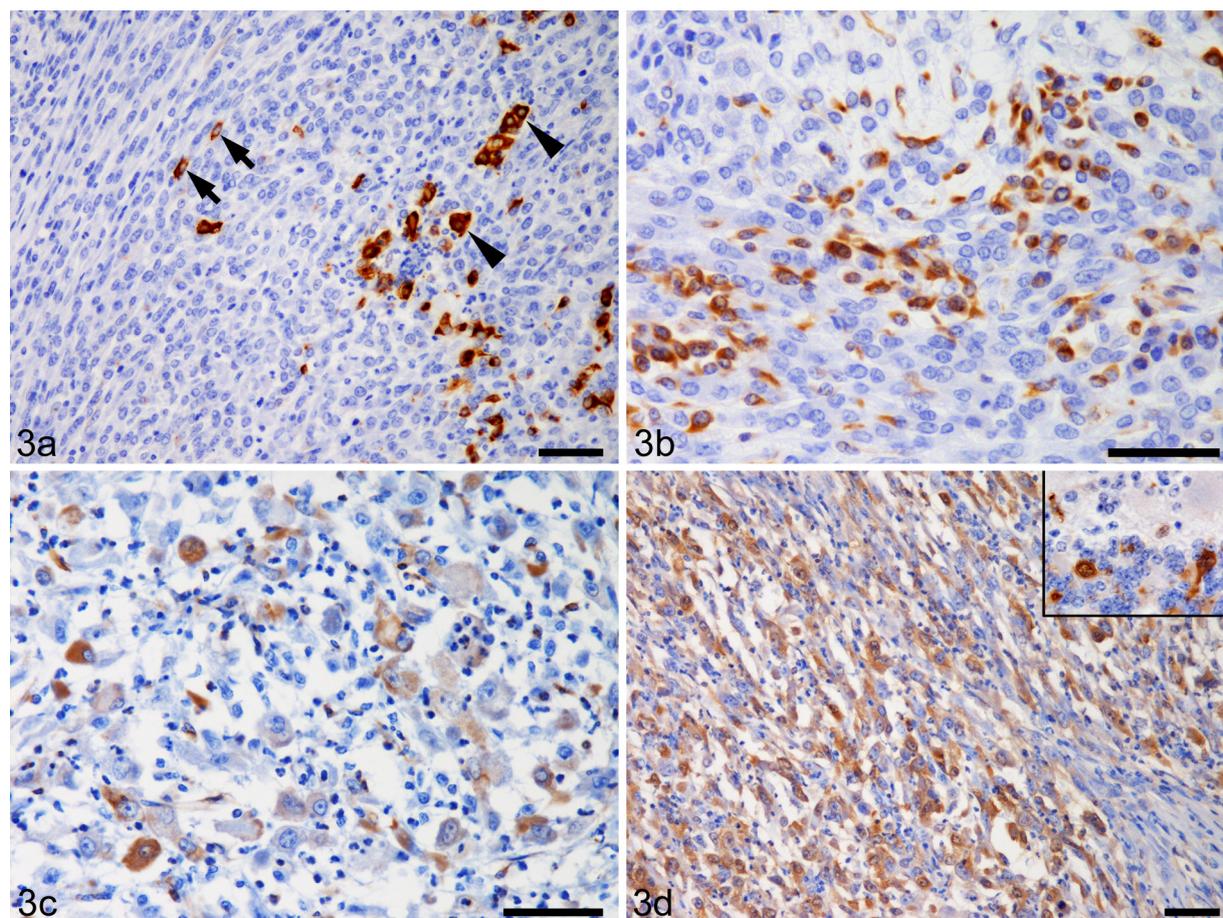
Fig. 3. (a) Epithelioid (arrowheads) and spindle-shaped (arrows) neoplastic cells immunohistochemically positive for pancytokeratin. Bar=50 μm. (b) Short spindle-shaped neoplastic cells immunohistochemically positive for vimentin. Bar=50 μm. (c) Epithelioid neoplastic cells immunohisto-chemically positive for vimentin. Neutrophils are scattered among the neoplastic cells. Bar=50 μm. (d) Spindle-shaped neoplastic cells immunohis-tochemically positive for calretinin. Bar=50 μm. Inset: The neurons in the cerebellum are immunohistochemically positive for calretinin.

vimentin, supports a diagnosis of mesothelioma. Calretinin is a calcium-binding protein, which is widely expressed in the central and peripheral nervous systems and is also expressed by normal and neoplastic mesothelial cells [18]. Calretinin is widely used as one of the most sensitive and specific markers of mesothelioma in humans, and recently, it was also used to diagnose mesothelioma in a horse and a striped skunk [8, 9, 17, 18]. As the antibody we used to detect calretinin specifically stains cerebellar neurons in sika deer, the positive staining of the neoplastic cells must be a reliable result and supports the diagnosis of mesothelioma made in the current case.

The differential histological diagnoses for sarcomatoid mesothelioma include sarcomas and sarcomatoid carcinomas [8, 11]. The immunohistochemical staining of cytokeratin allowed us to distinguish the present lesion from a sarcoma, which is usually negative for cytokeratin [8, 11]. Distinguishing sarcomatoid mesothelioma from sarcomatoid carcinomas can be difficult because both of these entities exhibit positive cytokeratin staining [8]; however, positive staining of vimentin, desmin, and α-SMA is less common in carcinomas. The positive staining of desmin and α-SMA was also reported in mesotheliomas that arose in a dog, cats, and humans, which might indicate the pluripotentiality of mesothelial cells [5, 7, 8, 13].

Based on the distribution of the lesions, the tumor in our case must have originated in the peritoneal cavity and metastasized to the pleural cavity. Invasive proliferating neoplastic cells and tumor thrombi were detected within the diaphragmatic muscle, and metastatic lesions formed on the pleural surface of the diaphragm and in the caudal mediastinal lymph nodes. From these findings, two potential pathways of tumor spread were considered as possible causes of the metastatic lesions in the pleural cavity. The first pathway involved the direct extension of the neoplasm from the peritoneal cavity into the pleural cavity through the diaphragm. The metastatic lesions on the pleural surface of the diaphragm might have formed via this pathway. The second pathway involved the tumor spreading from the peritoneal cavity to the pleural cavity through the diaphragm via the lymphatic system. Lymphatic drainage from the peritoneal cavity to the caudal mediastinal lymph nodes via the mediastinal lymphatic system has been demonstrated in cattle, sheep, and humans [1, 2, 14]. Therefore, the metastatic lesions that arose in the caudal mediastinal lymph nodes in the present case probably spread via the mediastinal lymphatic system.

In domestic animals, mesotheliomas are rare and most often occur in cattle, in which the peritoneum is the most frequent

location of such tumors [6, 11]. In deer species, only one case of pleural mesothelioma has been reported, which involved a European spotted fallow deer (*Cervus dama*, now reclassified as *Dama dama*) [4]. To the best of our knowledge, this is the first report about peritoneal mesothelioma in an animal belonging to the *Cervidae* family and the first reported case of mesothelioma in a sika deer.

In this report, we presented a case of mesothelioma in a sika deer. This case provides valuable knowledge about deer diseases. However, further information about the disease is needed to aid the treatment of sika deer and facilitate safety and hygiene inspections relating to venison production for human consumption.

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