Bilateral low grade serous adenocarcinoma of the ovaries in a badger (*Meles meles* L.) and its association with a borderline serous ovarian tumour: a case report

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ABSTRACT: Here, we describe a case of a wild female badger (a sow) with disseminated serous adenocarcinoma of the ovary which corresponds to a group of low grade serous carcinomas of the ovary in humans. Beside grossly apparent dissemination of the disease we observed a scale of histological features classifiable as a precursor lesion – borderline serous tumour of the ovary with implant metastases at the peritoneum, and features of the borderline tumour transformation in the carcinoma. The latter features included invasion of some of the metastatic peritoneal implants into the adipose tissue of the mesentery, retroperitoneum, and in the muscle of diaphragm with lymphangioinvasion and with blood-borne metastatic disease in the lungs. The primary tumour and its metastases had a uniform cytological appearance without atypia of the tumour cells. Mitotic activity was exceptional. The proliferation activity as demonstrated by immunohistochemical investigation of Ki-67 protein expression (revealing all active phases of the cell cycle – G1, S, G2, M) showed a low proliferation activity of the tumour cells, comparable with findings in low grade carcinomas or borderline tumours of the ovaries in women. WT1 protein was expressed in the whole tumour cell population. All these features were diagnostic of serous carcinoma of the ovary with low grade malignant potential. Tumours of the ovaries in wildlife have been described previously but they are infrequent and are rarely classified histopathologically. This case report offers a parallel with serous carcinomas in human pathology including features of transformation from a precursor lesion of a borderline serous tumour into a serous low grade carcinoma.

Keywords: *Meles meles*; ovary; borderline serous tumour; low grade serous carcinoma; metastases

In the Czech Republic as well as in many other countries limited attention has been paid to neoplastic diseases in wildlife mostly due to the difficulties in obtaining tissues for histopathological and molecular investigations. The frequency of neoplastic diseases in the Czech Republic in hares is estimated to be 1.7%, and in the fox population to be 1% (Bukovjan et al. 2011). In this report we describe a primary serous carcinoma of the ovary in a badger (*Meles meles*) disseminating into the peritoneal cavity and with metastatic spread into the lungs.

Various tumours of the ovaries were reported in wildlife. They occur sporadically in a variety of species ranging from amphibia to the mammals (Flux 1965; Bukovjan and Karpenko 1990; Munson and Montali 1991; Karpenko and Bukovjan 1996; Hofle et al. 2004; Fitzgerald et al. 2007). The finding of an ovarian carcinoma in a badger is unique and to the knowledge of the authors has yet to be reported.

Neoplasms in animals and humans share many morphological and biological features. Therefore, morphological evaluation of animal tumours and its interpretation may be applied in many cases in accordance with the International classification of tumours used in human oncology, ICD-O. We have followed the guidelines for classification of the re-

ported tumour with respect to analogous tumours in women.

Case description

Samples were taken from adult female badger (*Meles meles*) at the site of the Game and Hunt Association Pribyslav, district Havlickuv Brod, Czech Republic. The animal was found dead outside its burrow. At the initial inspection the badger was markedly cachectic (5.90 kg) and there was a moderate ascites. After opening the abdominal cavity the most conspicuous findings were enlarged ovaries measuring 6.2 × 3.9 × 3.5 cm and 5.7 × 3.1 × 2.8 cm. The ovaries were soft to spongy on palpation and on the cut section they had an irregularly microcystic appearance. There were multiple 1 mm- to 2 mm-sized grey nodules disseminated in the peritoneal cavity both on the serous surface of the parietal peritoneum and in the mesentery. The nodules were found also in the muscle of the diaphragm. The lungs were enlarged and oedematous. There were multiple small grey-white nodules up to 1 mm in diameter in the lung parenchyma. Other organs (liver, spleen, kidneys, heart, and uterus) were grossly unremarkable.

Gross samples taken from the primary tumour of the badger's ovaries and its metastases were fixed in 4% formaldehyde. The samples were subsequently dissected, embedded into paraffin blocks and processed according to a standard histological procedure at one of the authors' institutions (R.K.). Histological sections were stained with haematoxylin and eosin, mucicarmin and PAS with and without diastase digestion for the detection of mucin and glycogen. Immunohistochemical examination was performed with the aim of identifying the expression of intermediate filaments, WT1 protein and to measure the proliferative activity of the tumour using detection of the antigen Ki-67. The list of antibodies and antigen-demasking procedure is shown in the Table 1. The linkage of the primary antibodies was visualised using a two-step detection system (secondary biotinylated antibody, streptavidin-peroxidase complex; LSAB+ Dako Real detection system HRP/DAB+).

Microscopic evaluation revealed a neoplastic character of the disease with similar tumour tissue findings in the ovaries, lungs and in the peritoneal cavity. The ovarian tissue was almost entirely destroyed by the tumour. We found only focal residues of the ovarian stroma with single follicular cells (Figure 1). The tumour had mostly a cystopapillary arrangement but there were also foci of solidified neoplastic tissue with occasional tubular and cribriform features. The tumour cells had a cuboidal to low cylindrical shape, the cytoplasm

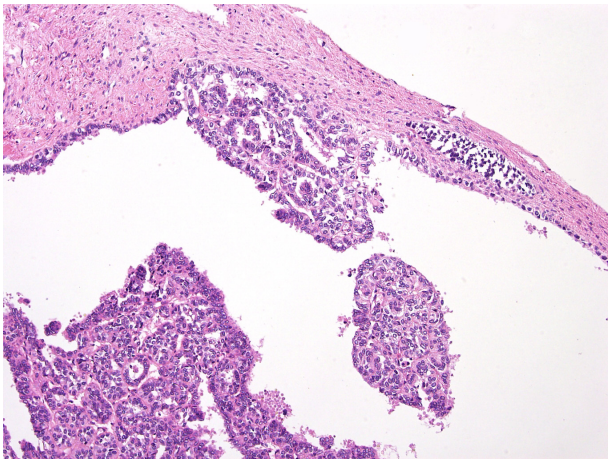

Figure 1. Primary tumour of the ovary. The ovarian tissue is preserved only in one small focus with follicular cells clustered in the ovarian stroma near the surface (upper right). The tumour has features of a low-grade papillary lesion of the ovary with a difficult diagnostic classification between a borderline serous ovarian tumour and a low-grade serous carcinoma; HE

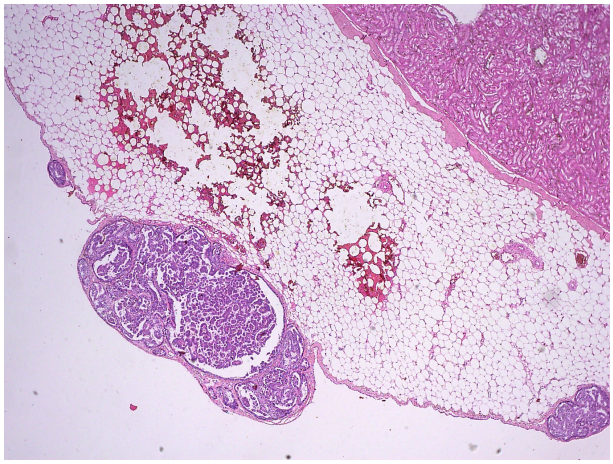

Figure 2. Non-invasive dissemination of the tumour into the parietal peritoneum. The tumour exhibits a microcystic papillary pattern of growth. It is sharply circumscribed from the retroperitoneal adipose tissue. Uninvolved kidney parenchyma can be observed upper right; HE

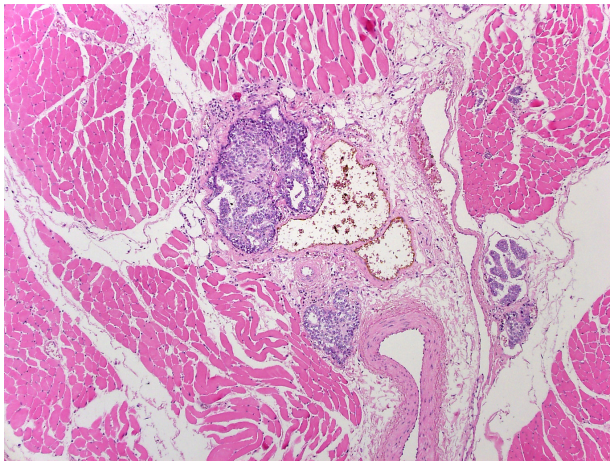

Figure 3. Invasive metastatic spread of the tumour in the diaphragm. Lymphatic spread of the tumour is seen on the left part of the picture; HE

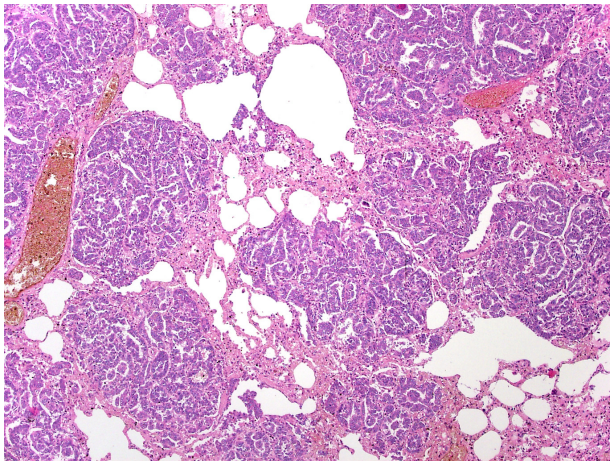

Figure 4. Lung metastatic spread in a form of partially coalescing tumour masses. The tumour maintains its cytological characteristics as well as some papillary features, but the tumour masses form also tubules and partially solidify; HE

was homogenous with a mild degree of basophilia, while staining for mucin and glycogen was negative. The nuclei of the tumour cells were relatively uniform, round with fine chromatin and mostly with a distinct central or paracentral nucleolus. Mitotic figures were rare. In a slender fibrovascular stroma without fibroplasia there were focal deposits of calcospherites. The metastatic deposits had a similar microscopic appearance when compared with the primary tumour. The metastases in the peritoneal cavity were observed both at the surface - non-invasive in the form of implants at the abdominal serosal surface (Figure 2), and some of them showed features of invasion into the fibrous and adipose tissue of the peritoneal cavity and also in the muscle of the diaphragm. Invasion into the lymphatic vessels was also observed (Figure 3). In the metastatic deposits in the lungs and focally in the diaphragm the tumour cells formed irregular tubular structures with slit-like lumina and also with solidified structures (Figure 4). The results of the immunohistochemical investigation are given in Table 1. The mixture of cytokeratins MNF116 showed a variable but in general moderately strong positivity of the tumour cells. Staining for vimentin was strongly positive in the entire tumour cell population, while desmin was negative with a positive internal control in the smooth muscle of blood ves-

Table 1. Antibodies used in immunohistochemical investigation on histological sections from paraffin-embedded tissues and the results

Antibody	Clone	Source/dilution/epitope demasking	Results – extent of positivity in the tumour cells/intensity of the staining
Anti-cytokeratins (CK 5, 6, 8, 17, probably 19)	MNF116	Dako/1 : 100/trypsin solution	focal to overall cytoplasmic/moderate
Anti-cytokeratin 7 (CK7)	OV-TL 12/30	Dako/1 : 100/boiling in buffer pH 9.0	overall/weak to moderate
Anti-vimentin	V9	Dako/1 : 100/boiling in buffer pH 6.0	overall positivity/strong
Anti-desmin	D33	Dako/1 : 100/boiling in buffer pH 6.0	negative (smooth muscle of vessels weak to moderate)
Anti-Wilms tumour 1 (WT1)	6F-H2	Dako/1 : 75/boiling in buffer pH 9.0	overall nuclear/moderate to strong
Anti-Ki-67 antigen	MIB-1	Dako/1 : 100/boiling in buffer pH 6.0	1–2%/moderate to strong

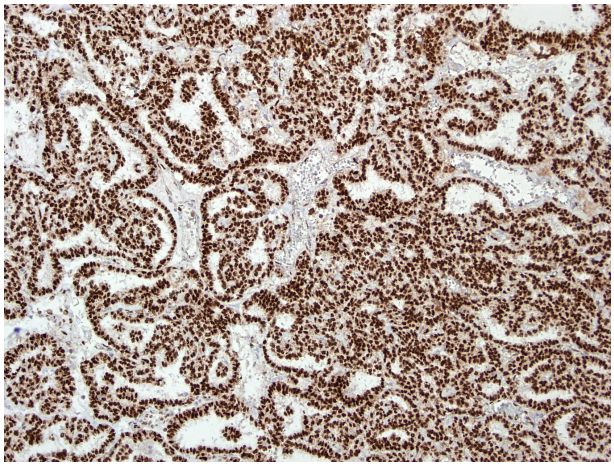

Figure 5. Moderate-to-strong nuclear staining of the WT1 protein in virtually the whole tumour cell population. The papillary structure of the tumour can be clearly discerned in this figure; anti-WT1 antibody, indirect immunoperoxidase method

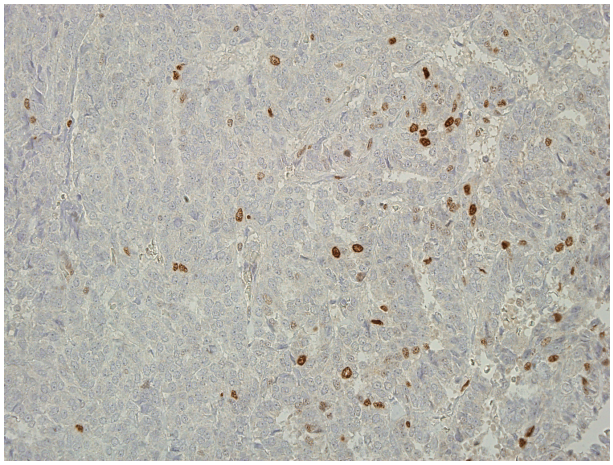

Figure 6. Ki-67 protein shows nuclear reactivity in occasional tumour cells, the majority being negative. The picture was taken from a cellular solidified region of the metastatic tumour in the lungs; anti-Ki-67 antibody, indirect immunoperoxidase method

sels. WT1 protein staining disclosed a moderate-to-strong nuclear positivity in the whole tumour cell population (Figure 5). Staining for Ki-67 protein in the primary tumour showed a moderately strong nuclear positivity in approximately 1% to 2% of the tumour cell population (Figure 6).

DISCUSSION AND CONCLUSIONS

Documentation and mapping of neoplasms in wildlife is important for comparison with tumours of domestic animals and also in seeking analogy with human pathology. In addition, such documentation facilitates understanding of the origin of animal tumours, their clinical behaviour and outcome, and in some instances may help in understanding disease aetiology (Mutinelli et al. 2004). According to the knowledge of the authors the described tumour is the first reported case of an ovarian adenocarcioma in a badger with histological and immunohistochemical specifications. The case also illustrates the relation between a low-grade serous ovarian carcinoma and a less aggressive precursor lesion, so-called borderline serous tumour of the ovary.

Ovarian tumours represent a heterogeneous group of diseases. Because the ovarian tissue has a developmentally complex cellular composition, classification reflects individual cell lines and is based on the histogenetic components. In a basic classification the ovarian tumours may originate from germinal cells – germ cell tumours, the second class of tumours has their origin in the cells of the ovarian stroma – gonadostromal tumors, and the third category includes tumours of the surface epithelium – epithelial and stromal tumours (Lee et al. 2003). In human pathology the epithelial tumours are most common. The biological behaviour of the epithelial tumours varies widely, from benign to highly malignant.

Epithelial ovarian tumours are further classified according to the differentiation of the tumour cell types. From these, serous adenocarcinomas, serous borderline tumours and a group of serous benign tumours are most frequent. Serous carcinomas represent 80% to 85% of all ovarian carcinomas. In an overwhelming majority of cases (about 95%) they are diagnosed at a late stage with spread into the peritoneal cavity beyond the pelvic peritoneum and/or with regional lymph node metastases (stage III), or with a distant metastatic spread (stage IV) (Seidman et al. 2004). In the described tumour of a female badger we observed a similar picture with primary bilateral carcinoma of the ovary with spread along the peritoneal cavity and a metastatic disease in the lungs. The tumour had features of a low-grade serous carcinoma, and was clearly distinguishable from a more frequent (at least in human pathology) high-grade serous carcinoma of the ovary which is characterised by cellular atypia and a high proliferative activity.

We believe that the low grade serous ovarian carcinoma in a badger is not only interesting *per se*,

but that also worthy of attention is the question of a possible progression from a precursor lesion of a borderline serous tumour of the ovary. Under the term borderline tumour of the ovaries we classify those lesions with signs of growth activity beyond the limits of a benign tumour but without features of a malignancy. The identification and precise classification of such cases facilitates the stratification of various grades of biological behaviour of epithelial tumours of the ovaries (Leitao et al. 2004). Borderline serous tumours are diagnosed mostly at stage I (70%), and they are in about one third of cases bilateral. Less frequently (30%) they are diagnosed at a stage of dissemination into the peritoneal cavity. In such cases it is necessary to distinguish this type of tumour from clearly invasive serous carcinomas of the ovary. A distinction between implantation non-invasive metastases typical for borderline tumours of the ovary and invasive metastatic spread which arises as either a primary feature of the carcinoma or as a reflection of progressive disease of borderline tumours and their transition into carcinomas is clinically important (McKenney et al. 2006; Silva et al. 2006). In cases of the presence of invasive implants the 10 to 20 year survival in woman drops to one third or to one half of the patients. As a sign of invasion the spread of implantation metastases into the adipose or muscle tissue underlying the peritoneal lining is considered diagnostic. Implantation metastases with cellular atypia or micropapillary endophytic growth patterns are considered as potentially invasive and are classified already within the low grade serous carcinoma. In the investigated badger the tumour was disseminated in the peritoneal cavity and part of the metastatic deposits had non-invasive features without cellular atypia. They fulfilled the criteria of diagnosis of disseminated borderline tumour. Beside that we observed metastases with micropapillary endophytic growth which fall into the category of potentially malignant implantation metastases, and also metastases with obvious invasion into the adipose tissue of the peritoneal cavity and into the muscle of the diaphragm, together with invasion into the lymphatic vessels. All these findings represent features of progression into the serous carcinoma. The malignant behaviour of the tumour was apparent also from the haematogenous spread of the tumour into the lungs.

Immunohistochemical investigation of these tumours was performed to confirm the epithelial nature of the tumour (also well apparent from the basic histological staining), and to specify the tumour cell kinetics as well as the differentiation line of the tumour cells. In epithelial tumours of the ovary in dogs the presence of keratins and also desmin was already demonstrated. Desmin positivity was reported by Akihara et al. (2007) and this finding was interpreted as proof of the origin of these epithelial tumours from the cells lining the coelomic cavity. In the present case we have observed a reactivity of cytokeratins MNF116 whereas desmin was negative, although there was desmin reactivity in the vascular smooth muscle as an internal control of the immunohistochemical results. Desmin negativity is not surprising because this protein is expressed in muscle cells and in some cases in mesothelial cells (in mesotheliomas). Thus, positivity in epithelial tumours of the ovary would be considered as aberrant rather than normal. The tumour cells in the described case were also strongly reactive for vimentin which was co-expressed with keratins. WT1 protein is a suitable additional marker for diagnosis. This protein is expressed in ovarian granulosa cells and also in the epithelial cells of the ovaries. WT1 reactivity has been demonstrated in some ovarian neoplasms, especially in serous carcinomas (Acs et al. 2004). Its expression in serous carcinomas is more frequently associated with high-grade carcinomas, and it may also have an adverse prognostic significance (Karamurzin et al. 2013). WT1 positivity in tumours of the ovaries may also be used in differential diagnostic evaluation to discriminate other carcinoma types (e.g. endometroid carcinoma) of the ovary (McCluggage 2011), and it may be helpful in evaluating the origin of a metastatic tumour – metastatic ovarian versus other metastatic carcinomas, for example from the mammary gland, from the stomach or large bowel (Tornos et al. 2005). However, WT1 protein is not entirely specific for ovarian tumours. It is expressed also in primary serous carcinomas of the peritoneal cavity and in some other tumours, which do not originate from the ovary or from the peritoneal lining.

Epithelial tumours of the ovaries have been described in dogs (Sforna et al. 2003; Hori et al. 2006; Akihara et al. 2007), in cattle (Yener et al. 2004) and in some other mammals. A similar observation to the reported case was described by Petterino et al. (2010) in a bitch. This tumour had according to the case description and photographs identical morphological and clinical findings with metastatic spread along the parietal and visceral peritoneum, to the pleural cavity and to the lungs. Beside cystopapillary structures there were psammomatous

bodies in the tumour stroma which were also observed in the present case though they were sporadic (Petterino et al. 2010).

Little is known about primary ovarian neoplasms in wild animals. This is probably due to the fact that limited attention is paid to animals by hunters and that autopsies are only rarely performed. Moreover, the pressure of predation in relation to a seriously ill and debilitated animal may also result in a falsely low estimate of neoplastic diseases in the wildlife population including the melinae. Debilitation of the organism together with tumour cachexia may also lead to death in a burrow without a possibility of identifying and investigating such an animal. In a tumour of the ovary (unilateral or bilateral) with implantation metastases along the peritoneum it is necessary to rule out the dissemination of neoplasms from other sites, for example from the uterus (Duncan et al. 2007). In human pathology there exists a rare primary carcinoma of the peritoneum without ovarian involvement which has the same morphological characteristics as its more common ovarian counterpart (Lee et al. 2003). Therefore, it is important to thoroughly inspect all organs at the post mortem investigation/evisceration.

The malignant neoplasm of the ovaries (code of ICD-O M-8461/3 C56.9) diagnosed in this adult female badger may be considered as a unique finding. Its morphological features and immunohistochemical profile is analogous to that seen in human pathology and includes morphological features indicative of progression from a borderline serous tumour of the ovary to an invasive and metastatic serous carcinoma.

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