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Multicentric peripheral T-cell lymphoma, bilateral granulosa cell tumor, and papillary ovarian adenocarcinoma in a female dog – case report

[*Linfoma multicêntrico de células T periférico, tumor de células da granulosa e adenocarcinoma papilar ovariano em uma cadela – relato de caso*]

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ABSTRACT

The frequency of ovarian neoplasms in dogs is low, and these tumors are often underdiagnosed mainly due to nonspecific clinical signs. Multiple ovarian tumors are rarely described in dogs and can be of primary or secondary origin. Lymphomas, in turn, are among the most common malignant neoplasms in dogs, with a variable clinical course. This report describes a case of multiple concomitant ovarian tumors and a multicentric lymphoma in a dog, diagnosed histologically and confirmed by immunohistochemistry. The right and left ovaries and mesenteric lymph node of a 14-year-old mixed breed female dog were received for histopathological evaluation. Histologically, a multicentric small to intermediate cell lymphoma was diagnosed in the lymph node and both ovaries, in addition to a bilateral granulosa cell tumor and papillary adenocarcinoma in one of the ovaries. Immunohistochemistry determined the diagnosis of multicentric peripheral T-cell lymphoma. Coexisting ovarian tumors are rarely described in the literature but can occur. This is the first report of these three neoplasms occurring concomitantly in the same organ. Therefore, clinicians and pathologists should be aware of this possibility when evaluating ovaries.
Keywords: multiple tumors; epithelial ovarian tumor; sex cord stromal tumor; multicentric lymphoma

RESUMO

A frequência de neoplasias ovarianas em cadelas é baixa, e esses tumores muitas vezes são subdiagnosticados, principalmente devido a sinais clínicos inespecíficos. Tumores ovarianos múltiplos são raramente descritos em cadelas e podem ser de origem primária ou secundária. Os linfomas, por sua vez, estão entre as neoplasias malignas mais comuns em cães, com curso clínico variável. Este relato descreve um caso de múltiplos tumores ovarianos concomitantes e um linfoma multicêntrico em uma cadela, diagnosticados histologicamente e confirmados por imuno-histoquímica. Os ovários direito e esquerdo e o linfonodo mesentérico de uma cadela, sem raça definida, de 14 anos foram recebidos para avaliação histopatológica. Histologicamente, diagnosticou-se linfoma multicêntrico de células pequenas a intermediárias em linfonodo em ambos os ovários, tumor de células da granulosa bilateral e adenocarcinoma papilar em um dos ovários. A imuno-histoquímica determinou o diagnóstico de linfoma periférico multicêntrico de células T. Tumores ovarianos coexistentes são raramente descritos na literatura, mas podem ocorrer. Este é o primeiro relato dessas três neoplasias ocorrendo concomitantemente no mesmo órgão. Portanto, clínicos e patologistas devem estar cientes dessa possibilidade ao avaliarem os ovários.

Palavras-chave: múltiplos tumores, tumor ovariano epitelial, tumor de células estromais sexuais, linfoma multicêntrico

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INTRODUCTION

Ovarian diseases are uncommon in dogs and can directly or indirectly affect the fertility and life of affected animals. In general, the low frequency is because females are usually spayed early (Arlt and Haimerl, 2016). The incidence of ovarian tumors in dogs varies from 0.5 to 6% (Banco *et al*., 2011; Arlt and Haimerl, 2016; Bhoi *et al*., 2022). However, according to Coggeshall *et al.* (2012), the real incidence is unknown because most studies are based only on organs from necropsy and/or biopsy, and not all organs removed surgically are submitted for such evaluation.

Primary ovarian tumors are classified according to their embryonic origin in: (1) epithelial tumors (which includes the surface epithelium of modified mesothelium, the rete ovarii, and subsurface epithelial structures); (2) germ cells; (3) and ovarian stromal cells (including sex cord stromal and gonadastromal elements). It is important to differentiate primary ovarian tumors from those that are metastatic or extend to the ovary. Ovarian tumors that include elements from more than one of the three lineages may occur, although rarely described, and must be differentiated between single tumors with more than one embryonic component and multiple tumors themselves (Agnew and Maclachlan, 2017).

Lymphoma is the most common malignant neoplasm of the hematopoietic system in dogs (Valli *et al*., 2016; Jark *et al*., 2020). The term lymphoma represents a variety of subtypes of lymphocytic neoplasms with distinct clinical characteristics and must be characterized according to the affected anatomical site, histopathological characteristics, and immunophenotype (Pittiway *et al*., 2019). According to a study by Jark *et al*. (2020), there is a higher risk for developing lymphoma in dogs older than seven years old in Brazil, and multicentric lymphomas are mainly of the B immunophenotype, with diffuse large B-cell lymphoma being the main type diagnosed.

This paper aims to describe a case of multiple concomitant tumors in the ovaries of a female dog, primary and secondary, histologically diagnosed and confirmed by immunohistochemistry.

CASUISTRY

The right and left ovaries and mesenteric lymph node of a 14-year-old mixed breed female dog were received for histopathological evaluation. According to the animal's history, the ovaries were removed after detection of increase in size during ultrasound examination. The reason for performing the ultrasound was not informed.

Grossly, the right ovary measured 4.5 x 3.3 x 2.7cm, with a soft to firm consistency. On the cut surface, the organ showed cystic cavities and solid areas, with a heterogeneous appearance and a yellow to brown color interspersed with brown areas. The left ovary measured 4.6 x 3.9 x 1.5cm, firm to soft in consistency. On cut surface the organ was solid, with a heterogeneous appearance and a light brown color with brown to yellow multifocal areas, in addition to a cystic cavity filled with translucent liquid. Finally, a mesenteric lymph node measuring 1.2 x 0.9 x 0.5cm, with a firm consistency, homogeneous and white on the cut surface, with brown areas, was sent for analysis. Fragments 3.0mm thick were selected, routinely processed, and stained with hematoxylin and eosin.

Histologically, the lymph node and both the right and left ovaries showed dense cellular neoplastic proliferation of small to intermediate-sized lymphocytes arranged in sheaths and extending to adjacent adipose tissue, causing loss of tissue architecture in the lymph node, as well as infiltration and bilateral expansion of the ovarian parenchyma (Figure 1A). Cells had scant and moderately delineated eosinophilic cytoplasm, round to oval, small to intermediate-sized nuclei, with dense chromatin and an inconspicuous nucleolus. The mitotic count was moderate, with about 30 mitoses in 2.37mm² in each ovary and about 40 mitoses in 2.37mm² in the lymph node. Among the neoplastic lymphocytes, a moderate number of apoptotic cells and occasional macrophages were observed.

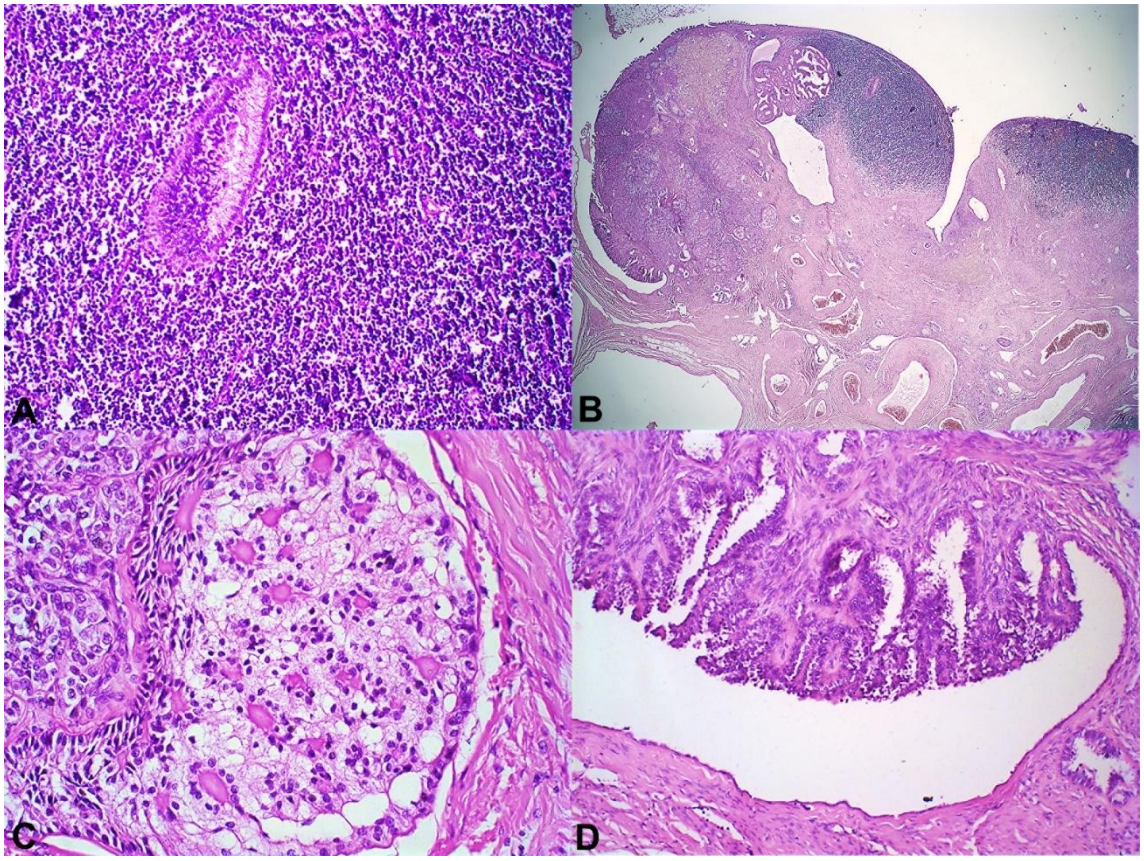

Figure 1. A. Right ovary with a dense cellular neoplastic proliferation of small to intermediate-sized lymphocytes arranged in sheaths, anchored in scant fibrovascular stroma (Hematoxylin and eosin, 200x). B. Left ovary with three neoplasms infiltrating and replacing the stroma (HE, 20x). C. Granulosa cell tumor diagnosed in both ovaries. Note the multiple Call-Exner bodies (HE, 400x). D. Papillary adenocarcinoma diagnosed in the left ovary (HE, 200x).

During the evaluation of the ovaries, two other neoplasms were noted, both primary, one of which bilateral (Figure 1B). The second neoplasm, diagnosed in both ovaries, was a granulosa cell tumor (Figure 1C). This presented similar histological characteristics in both organs, forming well-defined nodules with expansive growth, in a varied histological pattern, forming solid areas, cords and nests, rarely forming papillary areas. Cells were oval to elongated, with scant eosinophilic cytoplasm, poorly delimited, with oval nuclei and a prominent nucleolus. Cells were interspersed with a delicate fibrovascular stroma, some areas having a palisade arrangement, as well as forming radiated aggregates of tumor cells around a central deposit of strongly eosinophilic proteinaceous material, forming Call-Exner bodies (Figure 1C). There was moderate anisocytosis and anisokaryosis, and low mitotic activity in both ovaries (two mitotic figures in 2.37mm² in the left ovary and one in the right ovary).

The last neoplasm, observed only in the left ovary, was an ovarian papillary adenocarcinoma (Figure 1D). This was a proliferation of neoplastic cells forming arboriform papillary structures and occasional morulae supported by thin fibrovascular stroma. These were made up of carcinomatous epithelial cells supported by ramified stalks of connective tissue. Cells ranged from cuboidal to columnar, with ovoid nuclei and moderate eosinophilic cytoplasm. Moderate anisocytosis and anisokaryosis were noted, and two mitotic figures in 2.37mm².

The diagnosis of a round cell neoplasm compatible with small to intermediate cell lymphoma in both ovaries and in the mesenteric

lymph node, in addition to bilateral granulosa cell tumor and papillary adenocarcinoma in the left ovary, was achieved. To immunophenotypically characterize the lymphoma and confirm the diagnosis of multiple neoplasms in the left ovary, an immunohistochemical evaluation was recommended.

Multiple 3µm-thick sections were placed on gelatin slides and submitted to the immunohistochemical technique. Table 1 shows the antibodies used, the dilution, and the antigenic recovery method. All slides were labelled with 2-Methyl-4-isothiazolin-3-one (Novolink™ Polymer) and 3,3'-diaminobenzidine (DAB) was used the chromogen.

Table 1. Antibodies and immunohistochemical protocols applied

Target Antigen	Clone	Dilution	Antigen Retrieval Method
CD3	Polyclonal Rabbit Anti-human	1:200	Pressurized Heat (125°C/2min) with citrate buffer pH 6.0
Anti-CD20	Polyclonal Rabbit Anti-human	1:500	Pressurized Heat (125°C/2min) with citrate buffer pH 6.0
CD79a	Monoclonal Mouse Anti-human (HM47)	1:500	Pressurized Heat (125°C/2min) with citrate buffer pH 6.0
Pancytokeratin	Monoclonal Mouse Anti-Human Cytokeratin (AE1/AE3)	1:500	Pressurized Heat (125°C/2min) with citrate buffer pH 6.0
Ki67	Monoclonal Mouse Anti-Human (MIB-1)	1:50	Pressurized Heat (125°C/2min) with citrate buffer pH 6.0
Vimentin	Monoclonal Mouse Anti-Human (Vim 3B4)	1:500	Pressurized Heat (125°C/2min) with citrate buffer pH 6.0

Immunohistochemistry revealed lymphocytes positive for CD3 (Figure 2A) and negative for CD79 and CD20 both in the mesenteric lymph node and in both ovaries, thus configuring a multicentric peripheral T-cell lymphoma. Both ovarian papillary adenocarcinoma (Figure 2B) and granulosa cell tumor (Figure 2C) cells were intensely positive for cytokeratin; the labeling with vimentin was moderate in the granulosa cell tumor (Figure 2D) and weak on the adenocarcinoma and in the normal surface epithelium. The Ki67 antibody revealed rare positive nuclei both in the granulosa cell tumors and ovarian papillary adenocarcinoma.

DISCUSSION

This case describes multiple ovarian tumors, two primary and one multicentric, in a 14-year-old female dog. According to Agnew and Maclachlan (2017), multiple ovarian neoplasms can occur, although rarely observed. In the literature, there are few reports of different ovarian tumors in the same dog (Coggeshall *et al*., 2012; Oliveira *et al*., 2016; Oviedo-Peñata *et al*., 2020; Kita *et al*., 2022). The first case, described by Coggeshal *et al*. (2012), described a granulosa cell tumor and a teratoma in a one-and-a-half-year-old female dog. Subsequently, Oviedo-Peñata *et al*. (2020) described a similar case in a six-year-old female dog. Additionally, Oliveira *et al.* (2016) described a case of dysgerminoma and granulosa cell tumor in a six-year-old female dog. In the case described here, two primary ovarian neoplasms were observed, a bilateral granulosa cell tumor and an ovarian papillary adenocarcinoma, as well as a T-cell lymphoma, part of a multicentric disease. This is the first report of these three tumors in the same organ.

Granulosa cell tumors represent the main ovarian tumors diagnosed in dogs, representing more than 50% of neoplasms diagnosed in this organ, in this species, and originate in tertiary follicles (Patnaik and Greenlee, 1987). They usually occur unilaterally and eventually can be malignant, with metastatic capacity (Arlt and Haimerl, 2016). In the case described here, the tumor was small, without characteristics of malignancy, and bilateral. Additionally, in agreement with the literature, which reports that ovarian tumors are observed especially in intact aged female dogs (Oliveira *et al*., 2016), with a significant increase in the risk of developing tumors in dogs older than 10 years (Arlt and Haimerl, 2016), the patient in this case was an intact 14-year-old female dog.

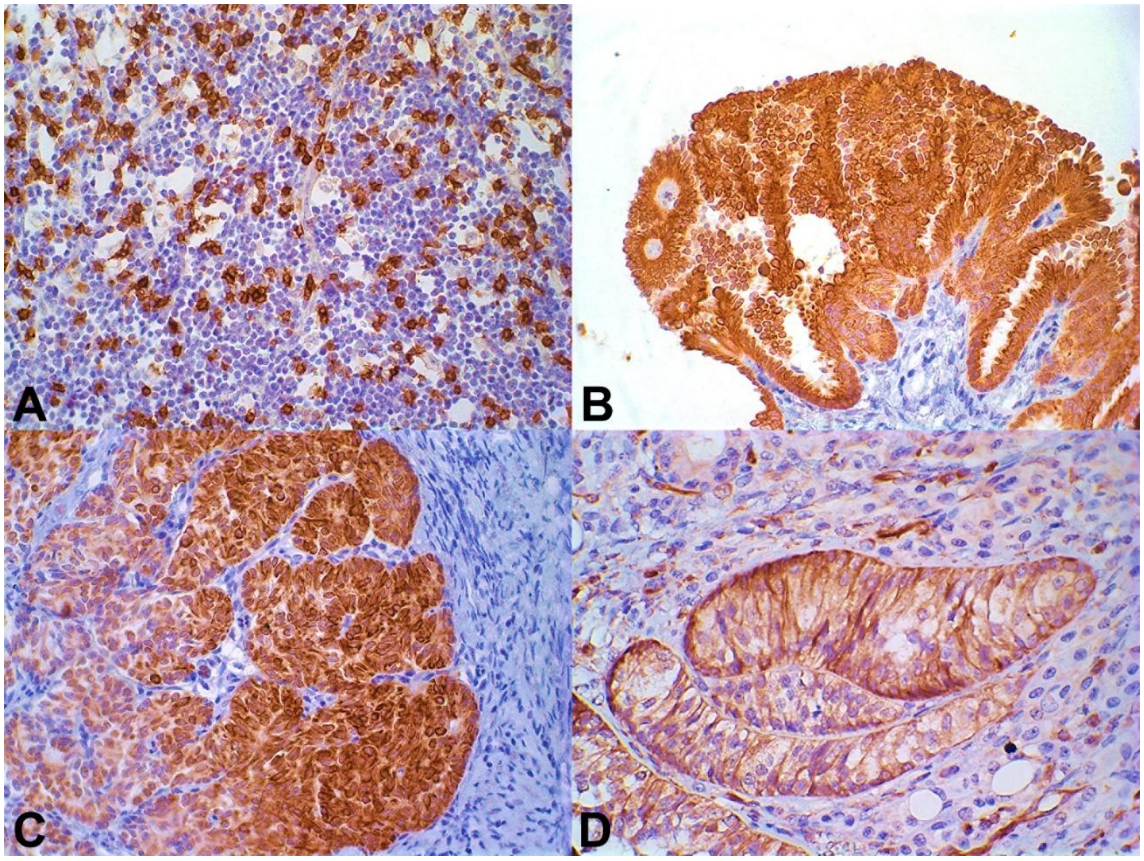

Figure 2. A. Multicentric lymphoma. Neoplastic cells are CD3 positive. B. Neoplastic cells in papillary adenocarcinoma are intensely positive for cytokeratin. C. Neoplastic cells in the granulosa cell tumor are intensely positive for cytokeratin. D. Neoplastic cells in the granulosa cell tumor are moderately positive for vimentin.

According to Oviedo-Peñata *et al*. (2020), granulosa cell tumors are often hormonally active since they originate from tertiary follicles. Clinically, affected animals may exhibit hyperstrinism, hyperestrogenism, masculinization syndrome, or persistent anestrus. However, due to limited clinical information available, it was not possible to determine the hormonal status of the female dog in this case, as well as possible clinical signs that led to the removal of the ovaries and mesenteric lymph node, in addition to the reported ultrasonographic alteration. Furthermore, eventually these tumors can exhibit malignant behavior, such as the presence of marked cellular pleomorphism, numerous mitotic figures, vascular invasion, and foci of necrosis and hemorrhage foci (Agnew and Maclachlan, 2017). Histologically, no features that would indicate malignancy were observed in the case described here.

In addition to the granulosa cell tumor, an ovarian papillary adenocarcinoma was diagnosed in one of the ovaries. These epithelial-originating tumors can, as granulosa cell tumors, be bilateral and usually appear as multiple papillary projections on the surface of the organ. They often present malignant behavior and dissemination by implantation/exfoliation in the abdominal cavity is common, a term called carcinomatosis. However, as observed in the case described here, the tumors may eventually be small and located only at the site of origin (Agnew and Maclachlan, 2017).

According to the literature, in the absence of carcinomatosis or evident vascular invasion, carcinomas should be diagnosed according to tumor size, presence of hemorrhage and necrosis, cell atypia, multiple layers of stacked cells, high mitotic count and, mainly, stromal invasion (Agnew and Maclachlan, 2017). In the case

described here, although part of the criteria was not observed, the presence of stromal invasion and multiple layers of carcinomatous cells were the criteria used for the diagnosis of adenocarcinoma. Additionally, due to the predominance of the papillary arrangement, the tumor was subclassified as papillary ovarian adenocarcinoma.

The granulosa cell tumors diagnosed in the case described here were positive for vimentin and cytokeratin, in agreement with what Riccardi *et al.* (2007), and ovarian adenocarcinoma was strongly positive for cytokeratin and weakly for vimentin. The use of these two markers, together with cytokeratin 7 and inhibin-α, is important mainly in cases in which the distinction between granulosa cell tumors and ovarian adenocarcinomas is not possible by routine histology, as the two tumors have different prognoses, the former having a lower probability of metastasis than the latter (Riccardi *et al*., 2007). In this case, both the granulosa cell tumors and the ovarian adenocarcinoma were well differentiated, and the applied markers aimed diagnostic confirmation. Furthermore, rare nuclei expressed Ki67 in both tumors, considering them negative for the marker according to Matos *et al*. (2021), who stated that positivity is considered when more than 25% of the nuclei are labelled.

According to the literature, the diagnosis of ovarian tumors in female dogs can be difficult due to discrete and nonspecific clinical signs, and they are often observed during elective or therapeutic procedures with other goals (Oviedo-Peñata *et al*., 2020), which may have been the case here. This fact is based on the advanced age of the patient, the size of the primary ovarian tumors, and the absence of vascular invasion or indications of carcinomatosis.

The dog in this case was also diagnosed, in addition to ovarian tumors, with a multicentric hematopoietic neoplasm. Lymphomas are among the main hematopoietic neoplasms diagnosed in dogs of different ages, and the multicentric form of this disease usually occurs in middle-aged to elderly animals (Valli *et al*., 2016). In general, large B-cell lymphoma is the main type of lymphoma diagnosed in dogs in Brazil, and only 27.1% of cases are classified as T-cell lymphomas (Jark *et al*., 2020). Furthermore, between 15 and 20% of all canine lymphomas are subclassified as multicentric peripheral T-cell lymphoma (Valli *et al*., 2016; Jark *et al*., 2020) as described in this case.

CONCLUSIONS

The frequency of ovarian neoplasms in dogs is low, and these tumors are often underdiagnosed. Coexisting ovarian tumors are rarely described in the literature but can occur. This is the first report of multicentric peripheral T cell lymphoma, papillary ovarian adenocarcinoma, and bilateral granulosa cell tumors that occur concomitantly in the same organ in veterinary literature. Therefore, clinicians and pathologists should be aware of this possibility when evaluating ovaries.

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