Braz J Vet Pathol, 2017, 10(3), 124 – 131
DOI: 10.24070/bjvp.1983-0246.v10i3p124-131

BJVP
Brazilian Journal of Veterinary Pathology
ISSN 1983-0246

Case Report

Intestinal mucinous adenocarcinoma with metastases to epididymis, testis and tunica albuginea in a dog

Danilo G. Wasques[1]*, Natália C. C. A. Fernandes[2], Rodrigo A. Ressio[2], Juliana M. Guerra[2]

[1]Histopet Anatomia Patológica, São Paulo, SP, Brazil.
[2]Instituto Adolfo Lutz, São Paulo, SP, Brazil.
*Corresponding author: Rua Moreira e Costa, 704. Ipiranga, São Paulo, SP. Zip Code: 04266-010, Brazil. E-mail: danilogw@gmail.com

Submitted March 18th 2017, Accepted May 24th 2017

Abstract

A case of intestinal mucinous adenocarcinoma with metastasis to gonadal tissue is reported. A 13-year-old, male, poodle dog presented with intestinal and peritoneal masses, as well as infiltrative masses in testicular tunics. Samples were biopsied and submitted for histopathological analysis. Microscopically, intestinal lesion consisted of an adenocarcinoma (mucinous type), with infiltration of muscular layers and mesenteric adipose tissue. In gonadal tissue, there was neoplastic infiltration of epididymis and tunica albuginea (with a predominantly tubular pattern), and testicular parenchyma (with a predominantly signet-ring cell pattern). Immunohistochemistry was positive for CDX2 and pancytokeratin, and negative for vimentin, supporting the diagnosis of intestinal mucinous adenocarcinoma with metastases to epididymis, testis and tunica albuginea.

Key words: immunohistochemistry, CDX2, testicular, epididymal, metastatic, canine.

Introduction

Intestinal adenocarcinoma is one of the most common malignant tumors in canine gastrointestinal tract, together with lymphoma and sarcomas (leiomyosarcomas and gastrointestinal stromal tumors) (14, 27). It presents generally with a high level of intestinal wall infiltration at the time of diagnosis, often with lymph node metastases, rendering a poor prognosis to the condition (25). An exception might be pointed out in cases of colorectal adenocarcinomas, which have a more insidious progression and longer survival rates (5). Diagnosis is generally made by histopathology, but immunohistochemistry with CDX-2 is an useful adjunct tool in equivocal cases, since it is an important specific marker of intestinal adenocarcinomas in humans, and has also shown immunopositivity in gastric and colorectal adenocarcinomas in dogs (10, 39).

Tumors in gonadal tissue are frequent in male dogs, almost exclusively affecting testis. These include Sertoli cell tumor, seminoma, interstitial cell tumor, mixed tumors, and, rarely, teratoma, gonadoblastoma, adenocarcinoma of rete testis, schwannoma and leiomyoma (17, 29). Although hyperplastic changes in epididymal epithelium are commonly noted (intraepithelial lumina and intraepithelial cysts), neoplastic disease is rarely reported (21). Mesothelioma is a rare tumor that may arise in tunica vaginalis testis (7, 38). Metastastic neoplasia affecting male gonadal tissue are rarely reported, and seems to be a rare or underdiagnosed event (1, 22, 35).

We report a case of intestinal infiltrative mucinous adenocarcinoma in a dog, with metastases to testis, epididymis and tunica albuginea, diagnosed by histopathology and supported by immunohistochemistry.

Case report

A 13-year-old, intact, male poodle dog presented with chronic emesis and weight loss, with intestinal and peritoneal masses, as well as infiltrative masses in testicular tunics detected on ultrasound. There were nodular lesions throughout intestine, with significant

 All rights reserved 2007.

Braz J Vet Pathol, 2017, 10(3), 124 – 131
DOI: 10.24070/bjvp.1983-0246.v10i3p124-131

luminal stenosis; a solid and irregular mass in peritoneum; and an infiltrative testicular lesion. Enterectomy and orchiectomy were performed, and two fragments from intestinal lesion and one testicle were submitted in 10% buffered formalin for histopathological analysis. Samples were routinely processed, sectioned at 5 µm, and stained with hematoxylin and eosin. Periodic acid-Schiff (PAS) and alcian blue special stains were also performed to evaluate mucin. For immunohistochemistry, after deparaffinization and hydration, antigen retrieval was carried on with citrate buffer 10 mM pH 6.0 in a pressure cooker for 3 minutes at 120ºC. Endogenous peroxidase was blocked with 6% hydrogen peroxide. Primary antibodies were incubated for 30 minutes at 37ºC, followed by overnight incubation (18 hours) at 4ºC. An extra slide set was incubated with PBS (pH 7.4), with 1% bovine serum albumin and 0.1% NaN_3, instead of primary antibody, as a control for nonspecific binding of secondary antibody. Human small intestine was used as positive tissue control, and human lymph node and tonsil were used as negative tissue controls. After incubation with post-primary (Post Primary, Max NovoLink Polymer Detection System, Novocastra, UK) for 30 min at 37°C, signal amplification with NovoLink (Novocastra) peroxidase short polymer system was carried on for 30 minutes at 37ºC. Development with 3,3'-diaminobenzidine 60 mg/mL (Sigma, St Louis M.O. Missouri, USA) on PBS pH 7.4 for 3 minutes at 37ºC was followed by counterstaining with Harris hematoxylin for 20 seconds at room temperature, dehydration and slide mounting with synthetic resin (Entellan Merck Millipore, Darmastadt, Germany). Monoclonal antibodies used included vimentin (V9 clone), 1:2000 (Invitrogen/Life Technologies Carlsbad, CA, USA), pancytokeratin (AE1AE3), 1:2000 (Biocare Medical, Concord, CA, USA), CDX2 (EPR2764Y clone), 1:500 (Cell Marque/Sigma Aldrich Company, St Louis M.O. Missouri, USA). For CDX2, replacement of primary antibody with rabbit IgG (1:500 dilution) was used as negative primary antibody control. Since pancytokeratin and vimentin reactions were done at the same dilution (1:2000), with antibodies from the same isotype and derived from the same species, and with different expected staining sites, each reaction was used as a negative antibody control for the other.

Grossly, intestinal samples submitted consisted of two tissue fragments, measuring about 2.5 x 1.5 x 2.3 cm, with smooth and whitish surface. These samples consisted partially of mesenteric adipose tissue and partially of a solid mural lesion, and mucosal surface was not detected**.** A testicle was also submitted, measuring about 2.5 cm in diameter, with a white nodule in the parenchyma, measuring 1.5 cm in diameter.

Microscopically, the sample from the intestinal mass lesion consisted of double layered smooth muscle tissue (consistent with intestinal muscle layers), extensively infiltrated by an epithelial cell population, arranged in tubular or microcystic structures, and less frequently as small nests or isolated cells, surrounded by marked fibrous reaction (Fig. 1A). There was frequently marked mucoid luminal secretion by neoplastic cells in tubular and cystic structures. Neoplastic cells had oval or irregular nuclei, with small prominent nucleoli, moderate anisokaryosis, and moderate amount of acidophilic cytoplasm. Rare mitotic figures were noted (0 to 1 mitotic figure per 400x high-power field). Most of the lesion consisted of cell-poor extravasated mucoid content amidst smooth muscle tissue. In some areas, neoplastic cells exhibited a signet-ring cell aspect, with peripheralized nuclei, and round mucoid-rich cytoplasm. There were focally extensive areas of infiltration of mesenteric adipose tissue by neoplastic tissue. The extra and intracellular material was PAS-positive and alcianophilic in special stains (Figs. 1D and 1G). Typical intestinal mucosa lining was not present in this sample

Samples from gonadal tissue showed extensive areas of neoplastic infiltration of epithelial cells in tunica albuginea and epididymal interstitium, predominantly with tubular to microcystic pattern, forming small nests (Figs. 1B and 2). In testicular parenchyma, there was diffuse infiltration of the interstitium by neoplastic cells, mostly with signet-ring cell morphology, and less often by tubular structures, intervening hypoplastic seminiferous tubules. There was mild effacement of testicular parenchyma, and the infiltrate mimicked the distribution of testicular interstitial cells (Fig. 1C). Mucoid material was also present intracellularly and in the lumen of tubular and microcystic structures, and were also positively stained by PAS and alcian blue special stains (Figs. 1E, 1F, 1H and 1I). Aside from malignant infiltration, two well-demarcated interstitial cell tumors without atypia were also present in testicular parenchyma.

At immunohistochemistry, neoplastic cells from adenocarcinoma showed a strong positive nuclear immunostaining for CDX2 (Figs. 1J, 1K and 1L), and strong membranous staining for AE1/AE3 (Fig. 3), both at intestinal and gonadal samples. Vimentin was negative in epithelial cells from adenocarcinoma, and positive in leydigomas, with positive internal control (vascular smooth muscle layer).

In a telephone call with the referring veterinarian, we were communicated that the animal died soon after surgery, but necropsy was not authorized by pet owners.

 All rights reserved 2007.

Braz J Vet Pathol, 2017, 10(3), 124 – 131
DOI: 10.24070/bjvp.1983-0246.v10i3p124-131

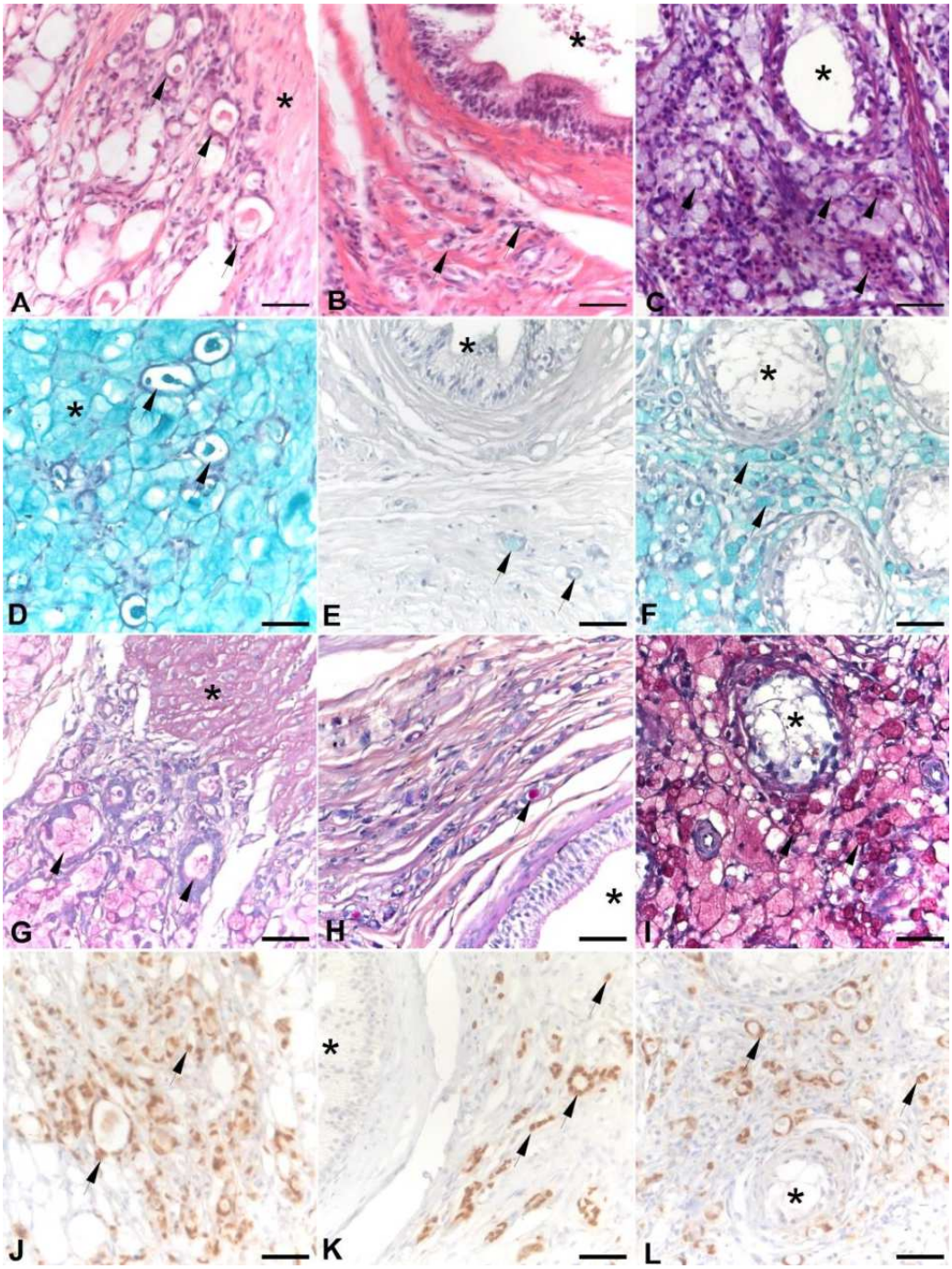


Figure 1. A. Intestine. Neoplastic tubules (arrows) infiltrating muscular wall (asterisk). HE. **B**. Epididymis. Small neoplastic acini (arrows) adjacent to epididymal duct (asterisk) HE. **C**. Testis. Diffuse infiltration of testicular interstitium by neoplastic cells with signet-ring cell morphology (arrows). Note pre-existing interstitial (leydig) cells (arrowheads). Seminiferous tubules are devoid of spermatogenic cells (asterisk). HE. **D**. Intestine. Abundant extracelular alcianophilic mucoid content (asterisk), with some neoplastic tubules interspersed (arrows). Alcian blue. **E**. Epididymis. Small neoplastic acini (arrows) containing alcianophilic luminal secretion, adjacent to epididymal duct (asterisk). Alcian blue. **F**. Testis. Signet-ring cells (arrows) with cytoplasmic alcianophilic content. Asterisk: seminiferous tubule. Alcian blue. **G**. Intestine. Neoplastic tubules with mildly PAS-positive luminal secretion (arrows) infiltrating muscular wall (asterisk). PAS. **H**. Epididymis. Small neoplastic acini (arrows) containing PAS-positive luminal secretion, adjacent to epididymal duct (asterisk). PAS. **I**. Testis. Signet-ring cells (arrows) with moderate to marked PAS-positive cytoplasmic staining. Asterisk: seminiferous tubule. PAS. **J**. Intestine. Marked nuclear staining of neoplastic epithelial cells (arrows). CDX2. **K**. Epididymis. Marked nuclear staining of neoplastic epithelial cells (arrows). Asterisk: epididymal duct. CDX2. **L**. Testis. Marked nuclear staining of neoplastic epithelial cells (arrows). Asterisk: seminiferous tubule. CDX2. Bars = 50 µm.

Braz J Vet Pathol, 2017, 10(3), 124 – 131
DOI: 10.24070/bjvp.1983-0246.v10i3p124-131

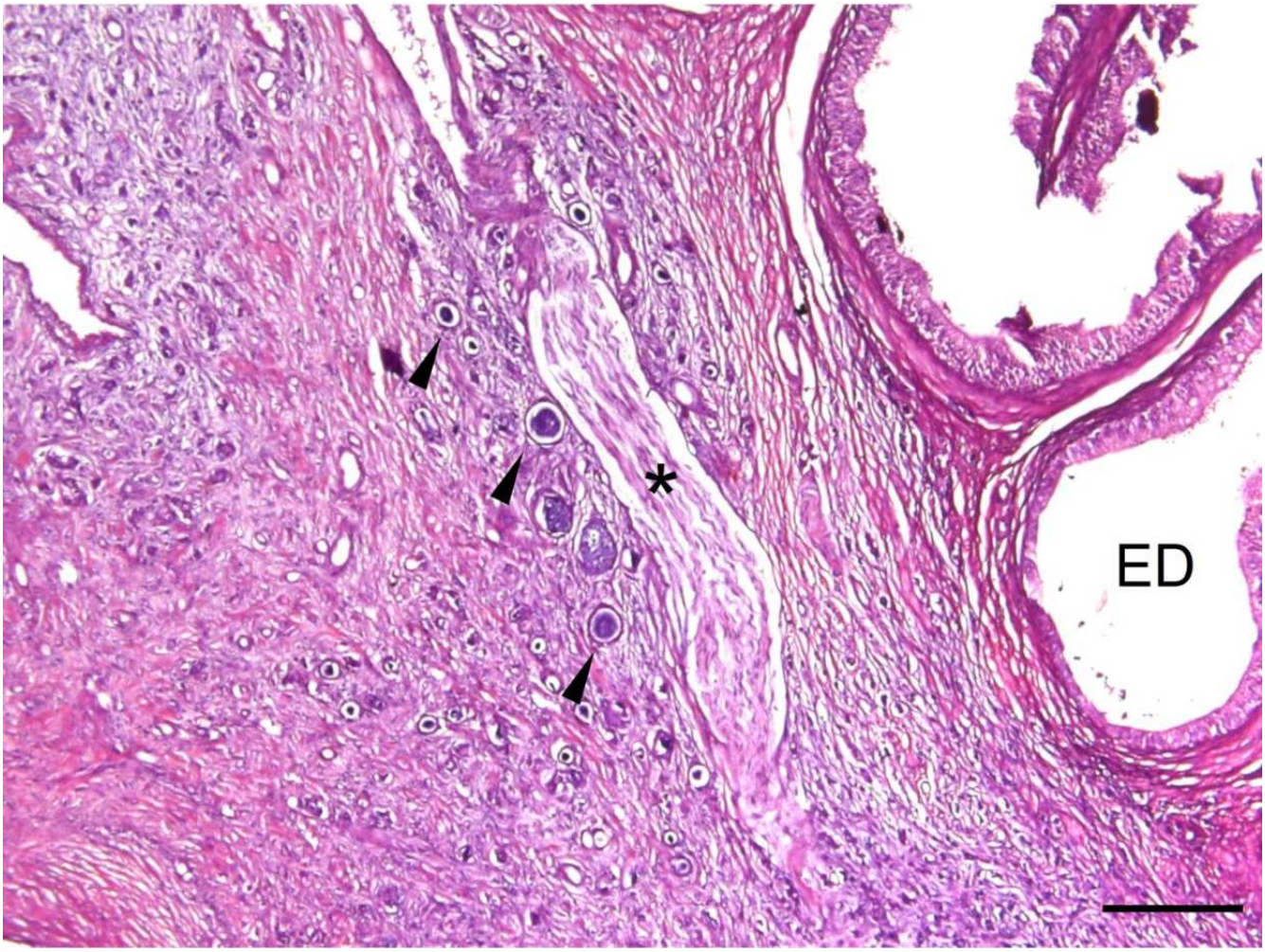


Figure 2. Epididimys and tunica albuginea. Infiltration of tunica albuginea and epididymis by neoplastic cells arranged in tubular pattern. Note perineural invasion. Asterisk: nerve. Arrowheads: neoplastic cells. DE: cross sections of ductus epididymis. HE. Bar = 200 µm.

Discussion

Adenocarcinoma is one of the most common intestinal neoplasms in dogs, and comprises 20% to 53% of intestinal tumors, and 37 to 55% of intestinal malignancies (14, 27). It carries a poor prognosis, and due to lack of specific clinical signs and late diagnosis, evidence of nodal or angiolymphatic invasion is noted in 52% and visceral metastases in 38% of cases at the time of diagnosis (25). Median survival after curative intent surgery has been reported to be 233 days (25).

Intestinal carcinomas may be classified in several distinct histological subtypes, based on the most predominant feature present. These subtypes include acinar/tubular adenocarcinoma, papillary adenocarcinoma, mucinous adenocarcinoma, signet-ring cell carcinoma, undifferentiated (solid) carcinoma and adenosquamous carcinoma (15). A prognostic significance of these different subtypes of carcinoma has not, however, been proven. The present report is an example of the morphological heterogeneity sometimes present in a neoplasia. In the primary site, morphology was consistent with a mucinous subtype of adenocarcinoma, with lakes of extracelular mucin predominating, with occasional neoplastic tubules intermingled. In gonadal tissues, different patterns were noted: in epididymis and tunica albuginea, tubular pattern predominated, and in testicular parenchyma, although some tubular structures were also noted, there was a predominance of signet-ring cell morphology, with an interstitial and diffuse pattern of infiltration. Interestingly, although neoplastic infiltration was obvious at tunica albuginea and epididymis, testicular infiltration was initially overlooked at scanning magnification, since the pattern of infiltration was reminiscent of interstitial cell hyperplasia. A cautious inspection at higher magnification and association with results from special stains with PAS and alcian blue confirmed the diagnosis of testicular infiltration of adenocarcinoma with a signet-ring predominant cell pattern in this organ.

The level of infiltration of intestinal adenocarcinoma has prognostic significance in humans,

Braz J Vet Pathol, 2017, 10(3), 124 – 131
DOI: 10.24070/bjvp.1983-0246.v10i3p124-131

with tumors that perforate visceral peritoneum and invade adjacent organs or mesentery having lower survival rates than those restricted to muscularis propria or submucosa (12). Grade has also residual prognostic significance for tumors that infiltrate beyond muscularis propria, with low-grade cases showing slightly better survival than high-grade ones (23). Similar associations have not yet been defined in veterinary literature. In the present case, the extensive areas of infiltration of mesenteric adipose tissue by neoplastic tissue were consistent with a neoplasia in an advanced stage and was expected to have a poor prognosis.

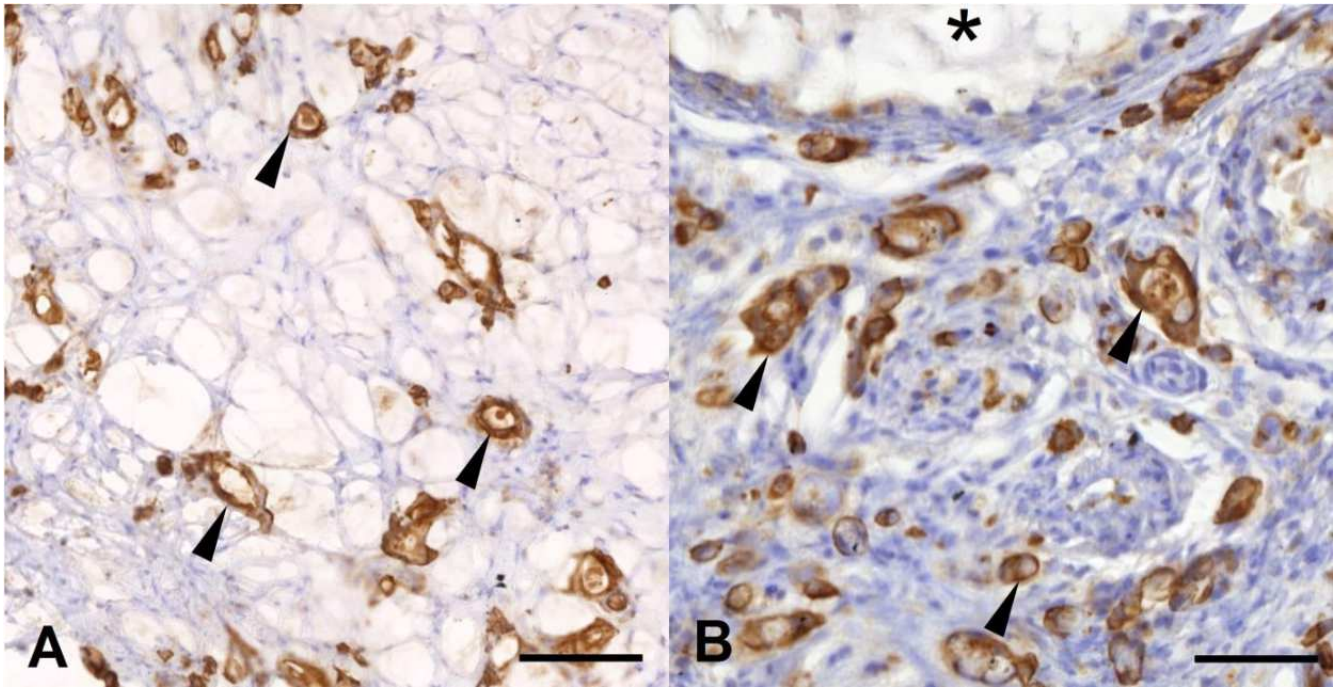


Figure 3. A. Intestine. Neoplastic epithelial cells showing marked membranous and cytoplasmic staining with pancytokeratin (AE1AE3). Immunohistochemistry. Bar = 100 µm. **B.** Testis. Neoplastic cells show membranous and cytoplasmic staining with pancytokeratin (AE1/AE3). Asterisk: seminiferous tubule. Immunohistochemistry. Bar = 50 µm.

Metastasis to gonadal tissues is rarely reported in veterinary literature. Some reported cases in dogs include testicular involvement with disseminated epithelioid hemangiosarcoma of unknown primary origin, disseminated histiocytic sarcoma and immunoblastic lymphoma (1, 22, 35). It is not known whether metastatic tumors to gonadal tissue are a rare or underreported occurrence in animals, and additional studies with a systematic evaluation of gonadal tissues in patients with disseminated neoplasia are needed to clarify this issue. In humans, metastatic lesions are reported to represent 1,6% to 7% of testicular tumors, with the most common origin being prostate carcinoma, and less common primary sites being renal cell carcinoma, transitional cell carcinoma (bladder and renal pelvis), seminal vesicle adenocarcinoma, lung adenocarcinoma, small cell carcinoma of the esophagus, colonic adenocarcinomas and carcinoid, melanoma, medulloblastoma, among others, and some are diagnosed as metastatic cancer of unknown primary site (11, 18, 37). We could not find reports of metastatic intestinal adenocarcinoma in gonadal tissue in veterinary literature, but this event is rarely reported in humans, with some of reported cases including colon adenocarcinoma metastatic to epididymis, occult gastrointestinal adenocarcinoma metastatic to testis, and cecum mucinous adenocarcinoma metastatic to tunica vaginalis testis (13, 32, 33). Some proposed routes by which colorectal carcinoma metastasize to testis include retrograde venous extension or embolism, retrograde lymphatic extension, arterial embolization, direct tumor invasion and retrograde spermiduct extension (13, 33). In the present report, a possible route for infiltration of gonadal tissues by neoplastic cells was by direct extension from primary site, since there was proven infiltration of neoplasia in visceral peritoneum and mesentery in the sample of the mass lesion, and there was also significant infiltration of tunica albuginea. Unfortunately, a necropsy could not be performed, and additional lesions that could offer clues to the mechanism of metastasis (e.g.: infiltration of inguinal canal, metastasis to other sites) could not be investigated.

 All rights reserved 2007.

Braz J Vet Pathol, 2017, 10(3), 124 – 131
DOI: 10.24070/bjvp.1983-0246.v10i3p124-131

An important differential diagnosis considered for this lesion was rete testis mucinous adenocarcinoma, as previously reported in one dog (29). Diagnostic criteria proposed for diagnosis of rete testis adenocarcinoma include: primary mediastinum testis involvement, no extension to parietal tunica, transition from normal rete testis to neoplastic epithelial cells, lack of any other neoplasia, lack of teratoma, and transmission electron microscopic findings (29). In the present case, a transition from normal rete testis to neoplastic rete testis was not evident, and neoplastic epithelial cells seemed to dissect pre-existing rete testis tissue. Furthermore, there was important involvement of intestinal tissue by the neoplasm, and neoplastic cells exhibited signet-ring cell morphology, occasionally described in gastrointestinal mucinous adenocarcinomas, but not in mucinous rete testis adenocarcinoma (although only one case was described) (29). Although there are some reports in human literature describing testicular tumors (seminomas, choriocarcinomas and teratomas) metastasizing to gastrointestinal tract, this hypothesis is unlikely in the present case, and the extension route from a primary intestinal tumor through parietal peritoneum and tunica vaginalis seems more plausible (2, 6, 8, 19, 34). Additionally, despite the fact that the samples examined did not reveal physical contiguity with intestinal mucosa, immunohistochemical expression of CDX-2 further supports the diagnosis of a neoplasia of intestinal origin.

An additional differential diagnosis to be considered in this case was gonadal infiltration of mesothelioma arising in tunica vaginalis. Mesotheliomas are classified as epithelioid (with several subclassifications, such as papillary, tubular, solid, desmoplastic, among others), sarcomatoid, and mixed/biphasic, and the epithelioid subtype was the one considered as a differential. Mucin production by neoplastic cells is a morphological criteria that should make mesothelioma less likely. Although there are some reports in human pathology describing mucin-producing mesothelioma, some even with signet-ring cell morphology, this is a rare event, and has been reported to occur only in approximately 5% of cases (20). Also, the immunohistochemistry profile of the present case (vimentin-, CDX2+) is not consistent with mesothelioma, which is reported to be vimentin positive in 100% of cases in dogs, and CDX2 negative in 100% of a series of cases of mesotheliomas with signet-ring cell features in humans (24, 28).

There is a current need for development of additional tools in veterinary pathology for the distinction between a primary and a metastatic tumor. In practice, it is not uncommon for pathologists to come across a case of a neoplastic lesion that is difficult, if not impossible, to classify as primary or metastatic on morphologic grounds alone. Correlation with clinical history and additional tests, such as imaging and endoscopy, is often needed in order to achieve a final diagnosis and, even so, some cases may remain undefined, and are considered metastatic cancer of unknown primary (31). For some tumors, markers with high specificity to the site of origin are available, such as uroplakin III (UPIII) for transitional cell carcinoma, thyroid transcription factor-1 (TTF-1) for primary lung tumors and CDX2 for gastrointestinal adenocarcinomas, but a comprehensive panel for accurately distinguishing different types of metastatic carcinomas has not been studied (30, 3, 10). In human pathology, different multiple-marker panels have been studied, including CDX2, PSA, TTF-1, CK7, CK20, among other markers, with reported accuracies varying from 65 to 75% in correctly predicting primary sites from carcinomas at metastatic sites (4, 9, 26). Nevertheless, metastatic cancer of unknown primary is still reported in human medicine (16). The more sensitive and specific a marker is, the more useful it is in diagnostic immunopathology. In this setting, CDX2 seems to be a promising tool in veterinary pathology for a more accurate diagnosis of metastatic adenocarcinomas. CDX2 is a transcription factor involved in regulation, proliferation, and differentiation of intestinal epithelial cells during development (10). Its expression has been reported in gastrointestinal tumors in dogs, with an estimated sensitivity of 84,6% for colorectal adenocarcinomas and 100% for gastric adenocarcinomas at primary sites, with positive staining also being achieved at metastatic site from one single case of metastatic gastric adenocarcinoma (10). Mammary gland adenocarcinomas, one hepatocellular carcinoma and renal cell carcinomas were negative for CDX2, suggesting it may also be a specific marker for gastrointestinal adenocarcinomas in dogs (10). On the other hand, a recent single case report has described expression of CDX2 in an urachal adenocarcinoma (36). Additional studies are currently needed to test also for specificity (i.e.: ruling out significant CDX2 expressions in other types of neoplasia, such as carcinomas from ovary, pancreas, biliary tree, lung, among others) and validate it as a candidate for a multi-panel used to diagnose primary neoplasia from metastatic sites. In humans, CDX2 has high sensitivity for colon adenocarcinoma, and less so for gastric adenocarcinomas, but it is not entirely specific, since it is also uncommonly to rarely expressed in extra-intestinal neoplasia, such as lung, ovarian, pancreatic and biliary tumors (9, 26, 38). In the present report, we were able to obtain a positive reaction for CDX2 not only at the primary focus, but also in gonadal metastasis, suggesting that CDX2 expression is preserved in metastatic cells. Ideally, additional studies should include samples from metastatic adenocarcinomas of different origins, to be tested blindly with CDX2, as well as with other putative specific markers to evaluate their predictive value in diagnosing sites of origin.

In conclusion, we report a case of intestinal mucinous adenocarcinoma with metastases to testis, epididymis, and tunica albuginea, with the aid of histochemical special stains and immunohistochemistry for pancytokeratin, CDX2 and vimentin. Pathologists should

 All rights reserved 2007.

be alerted that, albeit rare, gonadal tissue may be target for metastatic neoplasia. A careful and thoroughly evaluation of the whole slide should always be performed, and special care should be taken not to misinterpret or overlook the interstitial pattern of infiltration noted in testicular parenchyma. A special stain for mucin, such as alcian blue, together with immunohistochemistry may be invaluable in suspected cases. Proper differentiation of metastatic mucinous adenocarcinoma from mucinous adenocarcinoma of rete testis and mesothelioma should be done, when possible.

References

1. Affolter VK, Moore PF. Localized and disseminated histiocytic sarcoma of dendritic cell origin in dogs. Vet Pathol. 2002;39(1):74-83.
2. Armellino MF, Ambrosino F, Forner AL, De Stefano G, Robustelli U, Scardi F, Bellotti R, Calce R, De Stefano G. Jejunal perforation from metastatic choriocarcinoma. Case report and review of the literature. G Chir. 2008;29(4):145-8.
3. Bettini G, Marconato L, Morini M, Ferrari F. Thyroid transcription factor-1 immunohistochemistry: diagnostic tool and malignancy marker in canine malignant lung tumours. Vet Comp Oncol. 2009;7(1):28-37.
4. Brown RW, Campagna LB, Dunn JK, Cagle PT. Immunohistochemical identification of tumor markers in metastatic adenocarcinoma. A diagnostic adjunct in the determination of primary site. Am J Clin Pathol. 1997;107(1):12-9.
5. Church EM, Mehlhaff CJ, Patnaik AK. Colorectal adenocarcinoma in dogs: 78 cases. J Am Vet Med Assoc. 1987;191(6):727-30.
6. Cicin I, Ozyilmaz F, Karagol H, Yalcin F, Uzunoglu S, Kaplan M. Massive upper gastrointestinal bleeding from pure metastatic choriocarcinoma in patient with mixed germ cell tumor with subclinical intestinal metastasis. Urology. 2009;73(2):443.e15-7.
7. Cihak RW, Roen DR, Klaassen J. Malignant mesothelioma of the tunica vaginalis in a dog. J Comp Pathol. 1986;96(4):459-62.
8. Crookes PF, Cavallo AV, Balasubramaniam GS, Ryan P. Major gastrointestinal haemorrhage from a metastatic testicular teratoma. Aust N Z J Surg. 1992;62(8):657-9.
9. Dennis JL, Hvidsten TR, Wit EC, Komorowski J, Bell AK, Downie I, Mooney J, Verbeke C, Bellamy C, Keith WN, Oien KA. Markers of adenocarcinoma characteristic of the site of origin: development of a diagnostic algorithm. Clin Cancer Res. 2005;11(10):3766-72.
10. Doster AR, Yhee JY, Kim JH, Im KS, Sur JH. CDX-2 and HER-3 expression in canine gastric and colorectal adenocarcinomas. J Comp Pathol. 2011;145(1):12-9.
11. Dutt N, Bates AW, Baithun SI. Secondary neoplasms of the male genital tract with different patterns of involvement in adults and children. Histopathology. 2000;37(4):323-31.
12. Edge SB, Byrd DR, Compton CC, Fritz AG, Greene FL, Trotti A, editors. AJCC cancer staging manual. 7th ed. France: Springer; 2010. 648p.
13. Fahrner R, Theis B, Ardelt M, Rauchfuss F, Schüle S, Settmacher U. Rapid progressive colon cancer metastasized to the right epididymis and liver: report of a case and review of the literature. Int J Colorectal Dis. 2016;31(3):721-2.
14. Frgelecová L, Škorič M, Fictum P, Husník R. Canine gastrointestinal tract tumours: A restrospective study of 74 cases. Acta Veterinaria Brno. 2013;82(4):387-92.
15. Head KW, Cullen JM, Dubielzig RR, Else RW, Misdorp W, Patnaik AK, Tateyama S, Van Der Gaag I. Histological Classification of Tumors of the Alimentary System of Domestic Animals. Second Series. WHO, Armed Forces Institute of Pathology; Washington, D.C; 2003. 257p.
16. Karapetis CS, Guccione L, Tattersall MH, Gooden H, Vajdic CM, Lambert S, Robotin M, Mileshkin L, Schofield P. Perceptions of Cancer of Unknown Primary Site - A National Survey of Australian Medical Oncologists. Intern Med J. 2017;47(4):408-14.
17. Liao AT, Chu PY, Yeh LS, Lin CT, Liu CH. A 12-Year Retrospective Study of Canine Testicular Tumors. J Vet Med Sci. 2009;71(7):919-23.
18. Lu LY, Kuo JY, Lin ATL, Chang YH, Chen KK, Pan CC, Chang LS. Metastatic Tumors Involving the Testes. J Urol Roc. 2000;11(1):12-6.
19. Lund DM, Fode M, Balslev I, Sønksen J. Seminoma metastasis in the small bowel. Ugeskr Laeger. 2013;175(10):659-60.
20. McCaughey WT, Colby TV, Battifora H, Churg A, Corson JM, Greenberg SD, Grimes MM, Hammar S, Roggli VL, Unni KK. Diagnosis of diffuse malignant mesothelioma: experience of a US/Canadian Mesothelioma Panel. Mod Pathol. 1991;4(3):342-53.
21. McEntee K. Efferent ductules, epididymis, and deferent duct. In: McEntee K, editor. Reproductive Pathology of Domestic Mammals. San Diego: Academic Press; 1990. p.307-32.
22. Nieto JM, Pizarro M, Balaguer LM, Romano J. Canine testicular tumors in descended and cryptorchid testes. Dtsch Tierarztl Wochenschr. 1989;96(4):186-9.
23. O'Connell JB, Maggard MA, Ko CY. Colon cancer survival rates with the new American Joint Committee on Cancer sixth edition staging. J Natl Cancer Inst. 2004;96(19):1420-5.
24. Ordóñez NG. Mesothelioma with signet-ring cell features: report of 23 cases. Mod Pathol. 2013;26(3):370-84.

25. Paoloni MC, Penninck DG, Moore AS. Ultrasonographic and clinicopathologic findings in 21 dogs with intestinal adenocarcinoma. Vet Radiol Ultrasound. 2002;43(6):562-7.
26. Park SY, Kim BH, Kim JH, Lee S, Kang GH. Panels of immunohistochemical markers help determine primary sites of metastatic adenocarcinoma. Arch Pathol Lab Med. 2007;131(10):1561-7.
27. Patnaik AK, Hurvitz AI, Johnson GF. Canine gastrointestinal neoplasms. Vet Pathol. 1977;14(6):547-55.
28. Przeździecki R, Sapierzyński R. Using of immunocytochemistry in differential diagnosis of neoplasms of serosal cavities in dogs. Pol J Vet Sci. 2014;17(1):149-59.
29. Radi ZA, Miller DL, Hines ME 2nd. Rete Testis Mucinous Adenocarcinoma in a Dog. Vet Pathol. 2004;41:75-8.
30. Ramos-Vara JA, Miller MA, Boucher M, Roudabush A, Johnson GC. Immunohistochemical detection of uroplakin III, cytokeratin 7, and cytokeratin 20 in canine urothelial tumors. Vet Pathol. 2003;40(1):55-62.
31. Rossi F, Aresu L, Vignoli M, Buracco P, Bettini G, Ferro S, Gattino F, Ghiani F, Costantino R, Ressel L, Bellei E, Marconato L. Metastatic cancer of unknown primary in 21 dogs. Vet Comp Oncol. 2015;13(1):11-9.
32. Ruiz JM, Sanchis CM, López PC, Navarro HP, Moreno MJ, Sánchez AS, Tobarra MA, Rodríguez JA. Metastasis to the tunica vaginalis testis from a primary mucinous tumor of the cecum. Arch Esp Urol. 2010;63(3):235-8.
33. Salesi N, Fabi A, Di Cocco B, Marandino F, Pizzi G, Vecchione A, Cognetti F. Testis metastasis as an initial manifestation of an occult gastrointestinal cancer. Anticancer Res. 2004;24(2C):1093-6.
34. Senadhi V, Dutta S. Testicular seminoma metastasis to the gastrointestinal tract and the necessity of surgery. J Gastrointest Cancer. 2012;43(3):499-501.
35. Shor S, Helfand SC, Gorman E, Löhr CV. Diagnostic Exercise: Epithelioid Hemangiosarcoma Mimicking Metastatic Prostatic Neoplasia in a Dog. Vet Pathol. 2009;46:548-52.
36. Shrader S, Lauridson J, King Z, Loch J. Urachal Adenocarcinoma in a Dog. J Comp Pathol. 2016; 154(4):304-8.
37. Ulbright TM, Young RH. Metastatic carcinoma to the testis: a clinicopathologic analysis of 26 nonincidental cases with emphasis on deceptive features. Am J Surg Pathol. 2008;32(11):1683-93.
38. Vascellari M1, Carminato A, Camali G, Melchiotti E, Mutinelli F. Malignant mesothelioma of the tunica vaginalis in a dog. J Vet Diagn Invest. 2011;23:135-9.
39. Werling RW, Yaziji H, Bacchi CE, Gown AM. CDX2, a highly sensitive and specific marker of adenocarcinomas of intestinal origin: an immunohistochemical survey of 476 primary and metastatic carcinomas. Am J Surg Pathol. 2003;27(3):303-10.