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BILATERAL DYSGERMINOMA IN A SENILE BITCH ASSOCIATED WITH NODULAR LYMPHOID HYPERPLASIA IN SPLEEN AND PYOMETRA: A CASE REPORT

DISGERMINOMA BILATERAL EM CADELA SENIL ASSOCIADO A HIPERPLASIA NODULAR LINFOIDE EM BAÇO E PIOMETRA: RELATO DE CASO

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SUMMARY

Ovarian neoplasms affect a low percentage of female dogs, and these tumors are classified according to their cellular origin and are considered rare. Dysgerminoma is a tumor derived from undifferentiated primordial ovarian germinal epithelium cells. In the male, this neoplasm is diagnosed as seminoma. Thus, dysgerminoma is not linked to the production of ovarian hormones such as estrogen and progesterone, which play a fundamental role in the establishment of pyometra. At Araujo Veterinary Hospital (HVA), a 15-year-old female Pinscher was admitted with mucopurulent vaginal discharge, abdominal enlargement, anorexia, polydipsia, and prostration clinical signs indicative of pyometra, radiographic and ultrasound examinations were requested, as well as a blood collection for hemogram and serum biochemistry. Ultrasound examination revealed uterine enlargement and hypoechoic content, in addition to bilateral ovarian cysts, measuring 7 cm in the right ovary and 3 cm in the left. The blood count revealed normal values in the erythrogram and the leukogram showed monocytosis and lymphopenia, in addition to macroplatelets. Biochemical examination revealed increased GGT values of 10 U/L. Ovariohysterectomy was performed and, in the trans-surgical period, nodules in the spleen were observed. So, the ovaries and a fragment of the lymphoid organ, were fixed and sent to the histopathology laboratory after surgical resection. Histopathology revealed ovarian dysgerminoma and lymphoid nodular hyperplasia. Ten days after the surgical procedure, the dog returned to the HVA for clinical evaluation and suture removal. However, three days after the return, the patient presented a convulsive condition evolving to death.

KEY-WORDS: Ovarian neoplasm. Uterine infection. Splenic alterations.

RESUMO

Neoplasias ovarianas acometem baixa porcentagem de cadelas, e estes tumores são classificados de acordo com a origem celular, sendo considerados raros. O disgerminoma é um tumor derivado de células do epitélio germinativo primordial ovariano, indiferenciado. No macho, essa neoplasia é diagnosticada como seminoma. Assim, o disgerminoma não está ligado a produção de hormônios ovarianos, como o estrógeno e a progesterona, que desempenham papel fundamental para o estabelecimento da piometra. No Hospital Veterinário Araújo (HVA), uma cachorra da raça Pinscher, com 15 anos, foi antendida apresentando, secreção vaginal mucopurulenta, aumento abdominal, anorexia, polidipsia e prostração sinais clínicos indicativos de piometra, solicitou-se exame de radiográfico e ultrassonografia, bem como a coleta de sangue para hemograma e bioquímico sérico. O exame ultrassonográfico revelou aumento uterino e conteúdo hipoecóico, além de cistos ovarianos bilaterais, com mensurações de 7 cm no ovário direito e 3 cm no esquerdo. O hemograma revelou valores normais no eritrograma e o leucograma apresentou monocitose e linfopenia, além de macroplaquetas. O exame bioquímico revelou valores de GGT aumentados, em 10 U/L. A ovariohisterectomia foi realizada e no trans cirúrgico observou-se nódulos no baço. Assim, os ovários e um fragmento do órgão linfoide, após a ressecção cirúrgica, foram fixados e encaminhadas para laboratório de histopatologia. A histopatologia revelou disgerminoma ovariano e hiperplasia nodular linfoide. Dez dias após o procedimento cirúrgico, a paciente retornou ao HVA para avaliação clínica e retirada da sutura. No entanto, três dias após o retorno, o animal apresentou um quadro convulsivo evoluindo ao óbito.

PALAVRAS-CHAVE: Neoplasia ovariana. Infecção uterina. Alterações esplênicas.

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INTRODUCTION

Ovarian neoplasms affect a low percentage of female dogs throughout life and are considered rare (PARK et al., 2008). These tumors are classified according to their cellular origin in epithelial, germinal, stromal and mesenchymal (ROLIM et al., 2010). Dysgerminoma is derived from undifferentiated primordial ovarian germinal epithelium cells. In the male, this neoplasm is diagnosed as seminoma (McENTEE, 2002; ARLT & HAIMERL, 2016). Diez-Bru et al. (1998) and Novotny et al. (2011) report that dysgerminomas are diagnosed in 6 to 12% of cases.

Antonov et al. (2014) describe that this type of tumor affects 6 to 20% of female dogs with ovarian neoplasms. On Arlt and Haimerl (2016) study, in a total of 346 female dogs, dysgerminoma was diagnosed in 24, representing 6.93% of the cases. It is reported that dysgerminoma is not linked to the production of steroid hormones, such as progesterone (ARLT & HAIMERL, 2016), but granulosa cell tumors induce the secretion of them, related to the occurrence of cystic endometrial hyperplasia and pyometra (ZANGHI et al., 2007).

Pyometra is a uterine condition, commonly observed in the medical and surgical clinic of small animals, associated with acute or chronic suppurative bacterial infection with an accumulation of inflammatory exudate (DOW, 1959; HAGMAN, 2018). Bacterial infection of the uterus is called pyometra, and it is meaning in the veterinary medical literature is literally "uterus filled with pus" (EGENVALL et al., 2001). A common disease in intact adult female dogs and cats, is diagnosed less frequently in other small animal species (HAGMAN et al., 2014).

The involvement of ovarian hormones such as estrogen and progesterone plays a fundamental role in the establishment of the infection. Estrogen stimulates cell growth and endometrial cell vascularization, increasing sensitivity to progesterone (FIENI et al., 2014). Thus, under the action of progesterone, endometrial proliferation, glandular secretion, and decrease in myometrial contractions occur (SMITH, 2006). Furthermore, the primary hormonal imbalance or abnormal response to estrogen and progesterone concentrations affects uterine epithelial cells and facilitates adherence, colonization, and bacterial growth (FIENI et al., 2014; HAGMAN, 2018).

The increase in progesterone concentrations during estrus and initial diestrus, in female dogs, resulted in a reduction in nonspecific immunity, decreasing the expression of toll-like receptors (TLR) favoring embryo implantation and development (SILVA et al., 2012). The modulation of these receptors, which are pattern recognition, is responsible for initiating specific immune responses, and recruiting inflammatory cells such as granulocytes and neutrophils, favors colonization of the uterus by bacteria (HORNE et al., 2008).

The objective of this study was present a clinical case describing bilateral ovarian dysgerminoma with pyometra in a 15-year-old female Pinscher dog. In addition to clinical signs, sonographic and radiographic findings, hematological and biochemical data, surgical procedure, and findings, as well as confirmatory histopathological diagnosis are presented.

CASE REPORT

On the fourteenth of July, two thousand and twenty-two, a 15-year-old female Pinscher was admitted to the Araujo Veterinary Hospital (HVA) located in Jaú/SP with the following clinical signs: abdominal distension, mucopurulent vaginal secretion, polydipsia, inappetence for at least 3 days, lethargy, seborrheic skin, and prostration. The animal was obese and by abdominal palpation, the presence of nodules in the mammary gland chain was observed. During the clinical examination, it was possible to notice tracheal collapse. The observation of vaginal secretion was indicative of pyometra. A continuous act, an ultrasound examination was requested to confirm the diagnosis, as well as the collection of blood by venipuncture to perform a hemogram and serum biochemical analyses. This case report was approved by the University of Araraquara, UNIARA, Ethics Committee for the Use of Animals **RC001/21**

RESULTS OF COMPLEMENTARY EXAMS

The ultrasound examination (Figure 1) revealed a bilateral ovarian mass measuring 7 cm in the right ovary and 3 cm in the left (Figure 1A). In addition to uterine enlargement and hyperechoic structures in suspension, indicating the cellularity is characteristic of pyometra (Figure 1B). The blood count revealed normal values in the erythrogram (Table 1), in addition to macroplatelets from the spinal cord regenerative process. The leukogram (Table 2) showed monocytosis and lymphopenia, and blood cytology showed discrete erythrocyte rouleaux, that is, agglomeration of red blood cells, metarubricytes (2%), small platelet clusters, polychromasia, and discrete lipemia, in addition to 8 g/dL of protein plasma (reference value 6 – 8 g/dL).

Biochemical examination (Table 3) revealed a slight increase in albumin and total proteins. γ - glutamyl transferase (GGT). Values were increased by 10 U/L (1.2 – 6.4 U/L; THRALL, 2015). Furthermore, examination for free thyroxine (T4) revealed a dosage of 0.51 ng/dL slightly below the reference values (0.60 to 3.00 ng/Dl; THRALL, 2015). Such results allowed surgical resection of the uterus and ovaries.

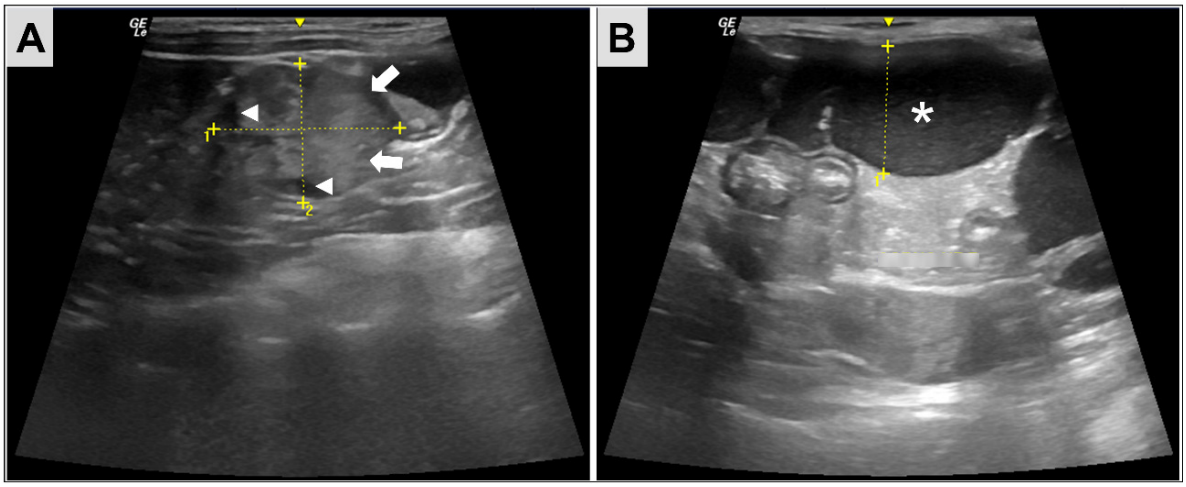

Figure 1 - Ultrasound examination of ovary and uterus. **(A)** Ultrasonography of the left ovary shows uniform echogenicity, spots hyperechoic (arrows), and some anechoic structures compatible with follicles (arrowhead). **(B)** The left uterine horn shows hypoechoic content, and hyperechoic structures in suspension, indicating the cellularity of the content present in the uterine lumen (*). Visualization of these structures associated with uterine distention is compatible with pyometra.

Table 1 - Blood count of a canine patient with pyometra and dysgerminoma.

Erythrogram	Value	Reference
Erythrocytes (x10^6/µL)	6.93	5.5 – 8.5
Hemoglobin (g/dL)	17.50	12 – 18
Hematocrit (%)	49.00	37 - 55
MCV[1] (fl)	71.90	60 - 77
MCHC[1] (g/dL)	35.10	32 - 36
Platelets (µL)	546.000	200 - 500.000

[1] MCV-Mean corpuscular volume; MCHC-Mean corpuscular hemoglobin.

Table 2 - Leukometric and total plasma protein values in a canine patient with pyometra and dysgerminoma.

White blood cell counts	Relative value (%)	Absolute value (µL)	Reference[1]
Total Leukocytes	12.700		6.000 – 17.000
Myelocytes	0	0	0
Metamyelocytes	0	0	0
Band neutrophils	1	127	0 - 300
Segmented neutrophils	74	9398	3.000 - 11.500
Lymphocytes	4	508	1.000 - 4.800
Monocytes	16	2032	150 - 1.350
Eosinophils	5	635	100 - 1.250
Basophils	0	0	rare

[1] Reference in absolute values of Thrall. (2015)

Table 3 - Serum metabolites in a canine patient with pyometra and dysgerminoma

Serum metabolites[1]	Absolute value (µL)	Reference[2]
Albumin (g/dl)	3.5	2.6 – 3.3
ALT (U/L)	49	21 - 102
ALP (U/L)	67	20 - 156
Total cholesterol (mg/dl)	216	125 - 270
Globulin (g/dl)	4.1	2.7 – 4.4
GGT (U/L)	10	1.2 – 6.4
Blood glucose (mg/dl)	104	70 - 110
Lipase (U/L)	46	13 - 200
Urea (mg/dl)	37	21.4 – 59.92
Creatinine (mg/dl)	1.1	0.5 – 1.5
Potassium (mmol/L)	5.3	4.37 – 5.35
Phosphorus (mg/dl)	4.6	2.6 – 6.8
Calcium (mg/dl)	10.2	8.6 – 11.2

[1] ALT (alanine aminotransferase); ALP (alkaline phosphatase); GGT (γ - glutamyl transferase)
[2] Reference in absolute values of Thrall. (2015)

SURGICAL THERAPY

The surgery was performed under general inhalation anesthesia with controlled mechanical ventilation. The animal received dipyrone (25 mg/kg – 0.22 mL), tramadol hydrochloride (4 mg/kg – 0.4 mL) and meloxicam (0.2 mg/kg – 0.5 mL) intramuscularly (IM) as pre-surgical drugs for pain control. For anesthetic induction, propofol (6 mg/kg – 2.6 mL) was used intravenously (IV) followed by intubation. The patient was maintained trans-surgical in an anesthetic plane with a mixture of 1.5% - 2% isoflurane and oxygen with a tidal volume of 10 - 20 mL/kg.

The surgical field was prepared, and the skin was cleaned and disinfected using a solution of 2% degerming iodine and 70% alcohol. Celiotomy was performed through a pre-retroumbilical incision in the linea alba, extending from the xiphoid cartilage to a few centimeters from the pubis. During exploratory celiotomy, nodules with a whitish smooth surface were observed in the spleen. A sample was collected for histopathological examination, and splenectomy didn't perform to preserve the patient because of the clinical character presented and advanced age. Then, the ovariohysterectomy was performed using the three-clamp technique as described by SLATTER, (2003). The spleen fragment and the ovaries were fixed in 10% buffered formalin solution, with pH 7.4, and sent for histopathological analysis with Hematoxylin and Eosin (H&E).

HISTOPATHOLOGICAL DIAGNOSIS

The histopathological diagnosis (Figure 2) of the ovaries showed a loss of normal bilateral ovarian architecture due to diffuse neoplastic proliferation (Figure 2A), consisting of strongly eosinophilic germ cells with ample cytoplasm, supported by rare bundles of delicate fibrous stroma. More than 20 mitotic figures per microscopic field (Figure 2B), in addition to rounded to oval nuclei with vesicular chromatin and conspicuous nuclei (0-3), marked anisokaryosis and pleomorphisms were observed, indicating malignancy. From these morphological analyses, it was concluded that it was compatible with ovarian dysgerminoma. The histopathological diagnosis (Figure 3) of the spleen revealed a nodular lesion covered by an intact splenic capsule, consisting of a proliferation of differentiated small lymphoid cells, a small number of blasts, and typical plasma cells compatible with nodular lymphoid hyperplasia.

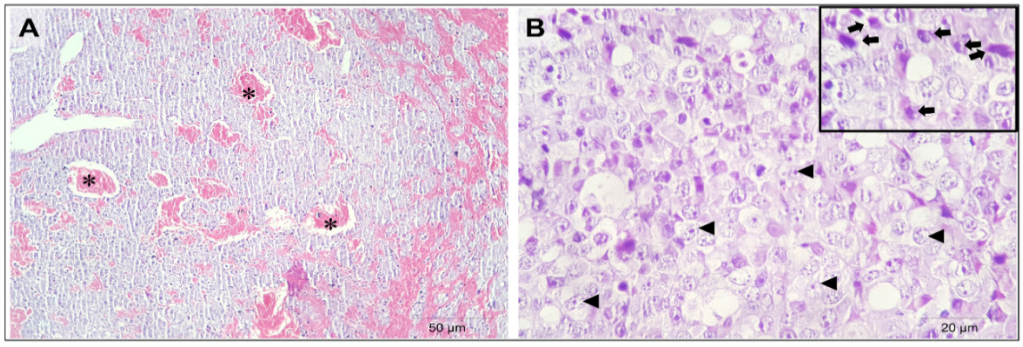

Figure 2 - Micrographs of ovarian dysgerminoma. (**A**) loss of normal ovarian architecture due to diffuse neoplastic proliferation, strongly eosinophilic germ cells (*) with imprecise boundaries forming extensive loose mantles and supported by delicate fibrous stroma (bar = 50 µm). (**B**) Conspicuous round to oval nuclei (arrowhead) with vesicular chromatin. (**B inset**) Marked anisokaryosis and pleomorphism (black arrow; bar = 20 µm). Hematoxylin and eosin (H&E).

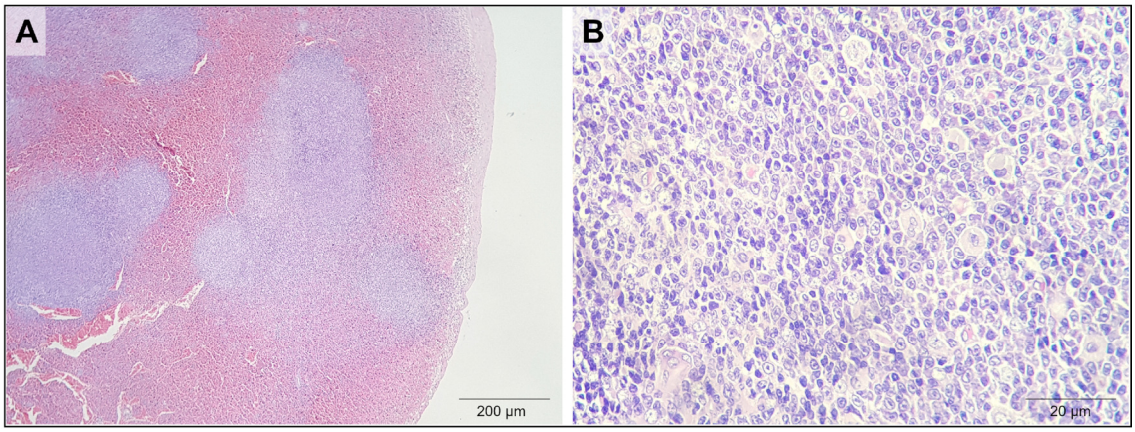

Figure 3 - Micrograph of lymphoid nodular hyperplasia. (**A**) Markedly dilated vascular channels filled with a large number of red blood cells (bar = 200 µm). (**B**) cells with minimal atypia (bar = 20 µm). Hematoxylin and eosin (H&E).

CONCLUSION OF THE CASE REPORT

The animal was hospitalized for two days at the HVA, and after clinical improvement, it was discharged. Ten days after the surgical procedure, the female dog returned for clinical evaluation, and sutures were removed, with no changes in the physical examination. However, three days later, the animal presented a convulsive condition evolving to death at tutors' home. Unfortunately, we don't know why the animal presented the convulsive condition, because the tutor informed that the animal came to die and didn't allow the necropsy technique. Therefore, wasn't possible to clarify if the death is related to dysgerminoma and/or pyometra.

DISCUSSION

This report described the diagnosis of open pyometra in association with bilateral dysgerminoma and lymphoid nodular hyperplasia (LNH), with a slight reduction in free thyroxine (T4) concentrations. The bilateral presentation of dysgerminoma associated with pyometra is reported as rare in only one article found in the literature (ROLIM et al., 2010). Given that, clinical symptoms such as abdominal enlargement, vaginal discharge, polydipsia, anorexia, lethargy, and prostration were clinical signs observed are compatible with those reported in dysgerminoma cases (ROLIM et al., 2010; NOVOTNY et al., 2011).

The clinical diagnosis of bitches with pyometra of the cervix, that is, when the cervix is open and with foul-smelling, bloody to mucopurulent vaginal discharge, is routinely performed. However, in the absence of vaginal discharge, associated with nonspecific clinical signs, this can be challenging (RENTON et al., 1993; PRETZER, 2008). In addition, other clinical findings such as lethargy, depression, inappetence, anorexia, polyuria, polydipsia, vomiting and diarrhea may also be present in patients with pyometra (PRETZER, 2008), which makes ovarian neoplasia diagnosis a diagnostic finding. A feature routinely found in dysgerminoma cases is the development of large, usually solid or lobulated, masses with hemorrhagic and necrotic areas (ARLT & HAIMERL, 2016).

Although the histopathological findings are similar in benign or malignant dysgerminoma, 10 to 20% of diagnoses show a malignant degree (McENTEE, 2002). Due to this, metastasis can be observed in lymph nodes, brain, liver, kidneys, adrenals, omentum, the surface of the serous intestinal tract, lungs, and retroperitoneal space (NOVOTNY et al., 2011; ANTONOV et al., 2014).

Reports are scarce in the literature related to diagnoses of dysgerminoma and lymphoid nodular hyperplasia (LNH). Sabattini et al. (2018) examined 35 dogs with NHL, and the mean age of the affected animals was 9.5 ± 2.8 years (4 to 17 years) being most representative breed being the Yorkshire terrier and other small breeds (≤10 kg). These data support the diagnosis of LNH, as the patient is small sized with advanced age (15 years). Little known about the biological behavior and development of lymphoid nodular hyperplasia (SABATTINI et al., 2018). However, the most common form of canine splenomegaly is LNH (non-neoplastic). The cells are usually found in the splenic parenchyma or resulting from regional neoplastic proliferation (benign or malignant) (SPANGLES & KASS, 1998).

The diagnosis of LNH is often obtained through abdominal ultrasound examination performed for other causes, such as staging of other unrelated tumors, ingestion of a foreign body, determination of pregnancy, enteropathy, cystitis, and a complete routine examination for chronic diseases (SABATTINI et al., 2018), or as in the present report in the transsurgical procedure through laparotomy. Survival data presented by Spangler and Kass. (1998) and Sabattini et al. (2018) point out that splenic proliferative nodular lesions (fibrohistiocytic cells considered malignant) related to grade I or II lesions (<60% of fibrohistiocytic cells) die within 12 months after splenectomy. Due to the patient's conditions and the clinical signs, as well as inappetence, lethargy and prostration aggravated by advanced age and diagnosis of pyometra. If affected by proliferative (malignant) nodular lesions, the female dog could die earlier. These data and patients' clinical conditions contribute to the understanding of the veterinary medical conduct in not performing splenectomy in the same surgical procedure as an ovariohysterectomy.

Although dysgerminoma does not contribute to the production of steroid hormones (ARLT; HAIMERL, 2016). Disorders in the bitch's estrous cycle may also be related to ovarian neoplasms originating in the stroma of the sex cords, causing hyperestrogenism (ROLIM et al., 2010).

In this context, estrogen stimulation before progesterone dominance is reported as a component of pathogenesis, with suppression of cellular immunity, resulting from an increase in progesterone concentration at the beginning of the luteal phase (SURGIURA et al., 2004). In diestrus (progesterone dominance), the secretory activity of the endometrial glands increases, as well as endometrial proliferation, decreases in myometrial contractility and cervix closure also occurs (SMITH, 2006; PRETZER, 2008; HAGMAN, 2018). Such conditions during this phase (diestrus) are reported to be favorable for bacterial colonization, even more, in combination with reduced local immunity by the action of progesterone and cumulative effects by repeated estrous cycles, evidence the increased incidence in middle-aged to older bitches (PRETZER, 2008; HAGMAN, 2018).

Although there is no correlation indicative of reduction or increase in thyroxine concentrations (free T4) associated with dysgerminoma, Thuroczy et al. (2016) report that in pregnant and non-pregnant female dogs, T4 concentrations are identical nonetheless, T4 levels are lower in process spontaneous abortion in which progesterone levels also rapidly decrease.

CONCLUSION

The exposed clinical case can be considered rare and aggravated by a uterine pathological condition such as pyometra, presenting clinical signs consistent with the clinical picture. However, the histological changes observed in the spleen and free thyroxine are neither worsened by the pathology nor predictive of the pathological change in the uterus. Dysgerminoma and lymphoid nodular hyperplasia can be found in older bitches, and in this specific case, it's not related to the death of the animal.

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