

# Intestinal T-cell lymphoma in an Asian small-clawed otter: case report and literature review of lymphoma in the subfamily *Lutrinae*

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**Abstract.** The Asian small-clawed otter (*Aonyx cinereus*) is an endangered species that is common in zoologic collections. A 17-y-old female Asian small-clawed otter under human care, with a clinical history of chronic renal disease, was euthanized because of deteriorating health. Histologically, the jejunal wall was infiltrated by a monomorphic population of small neoplastic lymphocytes that expanded the lamina propria of the villi and crypts, and on rare occasions invaded the submucosa. The tumor was composed of T cells (CD3+) with a proliferation index of 16%. Based on the World Health Organization (WHO) Classification of Hematopoietic Neoplasms in Domestic Animals, this lymphoma was classified as an enteropathy-associated T-cell lymphoma (EATL) type 2. We also present here a review of the literature on intestinal lymphoma in the subfamily *Lutrinae* (otters).

**Keywords:** enteropathy-associated T-cell lymphoma; intestine; lymphoma; mustelids, otters.

The Asian small-clawed otter (*Aonyx cinereus*, *Mustelidae*, *Lutrinae*) is a small semiaquatic mammal autochthonous to Southeast Asia. This otter species was categorized as vulnerable in the International Union for Conservation of Nature (IUCN) Red List.<sup>19</sup> Furthermore, the Asian small-clawed otter is listed in Appendix I of the Convention on International Trade in Endangered Species (CITES) from 2019.<sup>14</sup> *A. cinereus* is relatively common in zoologic collections.<sup>11,14</sup>

The most common pathologic condition in Asian small-clawed otters is nephrolithiasis.<sup>10,20</sup> Infectious diseases such as infection by canine parvovirus 2a (*Carnivore protoparvovirus 1*),<sup>14</sup> or canine parvovirus 2b and *Clostridioides difficile* coinfection,<sup>16</sup> have been reported. Dirofilariosis (*Dirofilaria immitis*),<sup>15</sup> visceral pentastomiasis,<sup>3</sup> osteoporosis,<sup>5</sup> and cyanide toxicosis caused by ingestion of loquat (*Eriobotrya japonica*),<sup>18</sup> have also been described.

Reports of neoplasia in Asian small-clawed otters are scarce; most cases are reported in aged animals.<sup>12</sup> Bone-invasive lymphoma leading to paraplegia<sup>12</sup> and functional thyroid carcinoma with metastasis to lymph nodes (LNs) have been described in Asian small-clawed otters.<sup>3</sup>

Lymphoma is a relatively common tumor in the order *Carnivora*, both in domestic and wild species, but are reported uncommonly in family *Mustelidae*—except in domestic ferrets (*Mustela putorius furo*).<sup>11</sup> Lymphoma has been reported only rarely in the subfamily *Lutrinae* (Table 1).

A 17-y-old female Asian small-clawed otter from a zoologic collection, with a clinical history of a chronic renal disease, was euthanized because of worsening health status. A postmortem examination was performed immediately after

death. The main gross finding was severe bilateral nephrolithiasis with hydronephrosis. Other gross lesions included poor body condition, loss of incisors, mineral deposits in the visceral pleura, and severe, chronic, diffuse, hypertrophic and hemorrhagic gastritis. The jejunum was diffusely thickened. Intestinal serosa and mesenteric lymphangiectasia and mesenteric lymphadenomegaly were noted (Fig. 1A). There was no evidence of intestinal perforation or peritonitis.

Samples of the main organs and tissues were collected for histologic study. Tissue samples were fixed in neutral-buffered formalin for 48 h, processed routinely, and sections stained with H&E. Histologically, in sections of jejunum, a poorly demarcated, non-encapsulated, densely cellular neoplasm composed of monomorphic lymphocytes effaced the lamina propria of the mucosa, and occasionally infiltrated the submucosa. These neoplastic lymphocytes were arranged in a band that expanded the villus-crypt junction and infiltrated the villi, the crypts, and, on rare occasions, the submucosa (Fig. 1B). Stroma was scarce. Neoplastic lymphocytes had small, round, centrally placed nuclei of 6–8- $\mu$ m (1.5

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**Table 1.** Summary of the published case reports of lymphoma in the subfamily *Lutrinae* (otters), including species, location, and immunophenotyping.

Otter species	Organ(s) affected	Neoplastic lymphocytes	Pleomorphism	Mitoses/hpf	Immunophenotyping	Ref.
Asian small-clawed otter ( <i>Aonyx cinereus</i> )	Spleen, LNs, liver, kidney	Large	Mild	Low	B cell, CD79 $\alpha$ +	11
Eurasian otter ( <i>Lutra lutra</i> )	Spleen, LNs, thymus, adrenal glands	NS	Mild, monomorphic	NS	NS	2
	Intestine	Large, granular cells	Mild, monomorphic	5–6	T cell, CD3+	1
North American river otter ( <i>Lontra canadensis</i> )	Spleen	Large (2–2.5 $\times$ RBC)	Mild	2–3	T cell, CD3+	6
Sea otter ( <i>Enhydra lutris</i> )	LNs, thymus, liver	Large, lymphoblastic	Mild	0–3	NS	5
	Brain	Small	Moderate	High, PCNA+	T cell, CD3+	13

LN=lymph node; NS=not specified; PCNA=proliferating cell nuclear antigen.

RBCs) diameter, with vesicular chromatin, a single small nucleolus, and scant, lightly eosinophilic cytoplasm. There was mild anisocytosis and anisokaryosis. The mitotic count was 10 in 2.37 mm<sup>2</sup> (10 contiguous hpf, 40 $\times$  objective, 10 $\times$  ocular field number 22 mm). Additionally, there was severe villus blunting and fusion, multifocal erosions of the villi, and moderate edema of the lamina propria and submucosa.

Cortical atrophy and bilateral suppurative pyelonephritis were observed in urolith-containing areas of the kidney. Renal areas not affected by urolithiasis displayed changes consistent with an end-stage kidney. Non-renal lesions of uremia, such as metastatic foci of mineralization, and moderate diffuse hyperplastic-hemorrhagic gastritis, were observed. Other findings included cystic endometrial hyperplasia, and lymphoid atrophy in the spleen and LNs.

Intestinal paraffin sections were placed on charged slides, deparaffinized in xylene, and rehydrated in an ethanol series. After heat-induced epitope retrieval using a pressure cooker with citrate buffer pH 6 (Panreac Química), the samples were incubated in a hydrogen peroxide solution in methanol (Panreac Química) to quench endogenous peroxidase. The samples were then incubated in horse serum followed by primary antibodies: rabbit polyclonal anti-human CD3 (clone UCHT1, 1:200; Dako, Agilent), mouse monoclonal anti-human CD79 $\alpha$ cy (clone HM57, 1:50; Dako, Agilent), and rabbit monoclonal anti-human Ki-67 (clone SP6, undiluted; Master Diagnóstica) antibodies. Commercial reagents were used for the secondary antibody (ImmPRESS-VR horse anti-rabbit IgG polymer kit; Vector) and chromogen (ImmPACT NovaRED peroxidase substrate; Vector). Spleen from an unrelated otter was used as a positive control. For negative controls, the primary antibody was replaced by a commercial universal negative control reagent. Finally, samples were counterstained with hematoxylin and coverslipped.

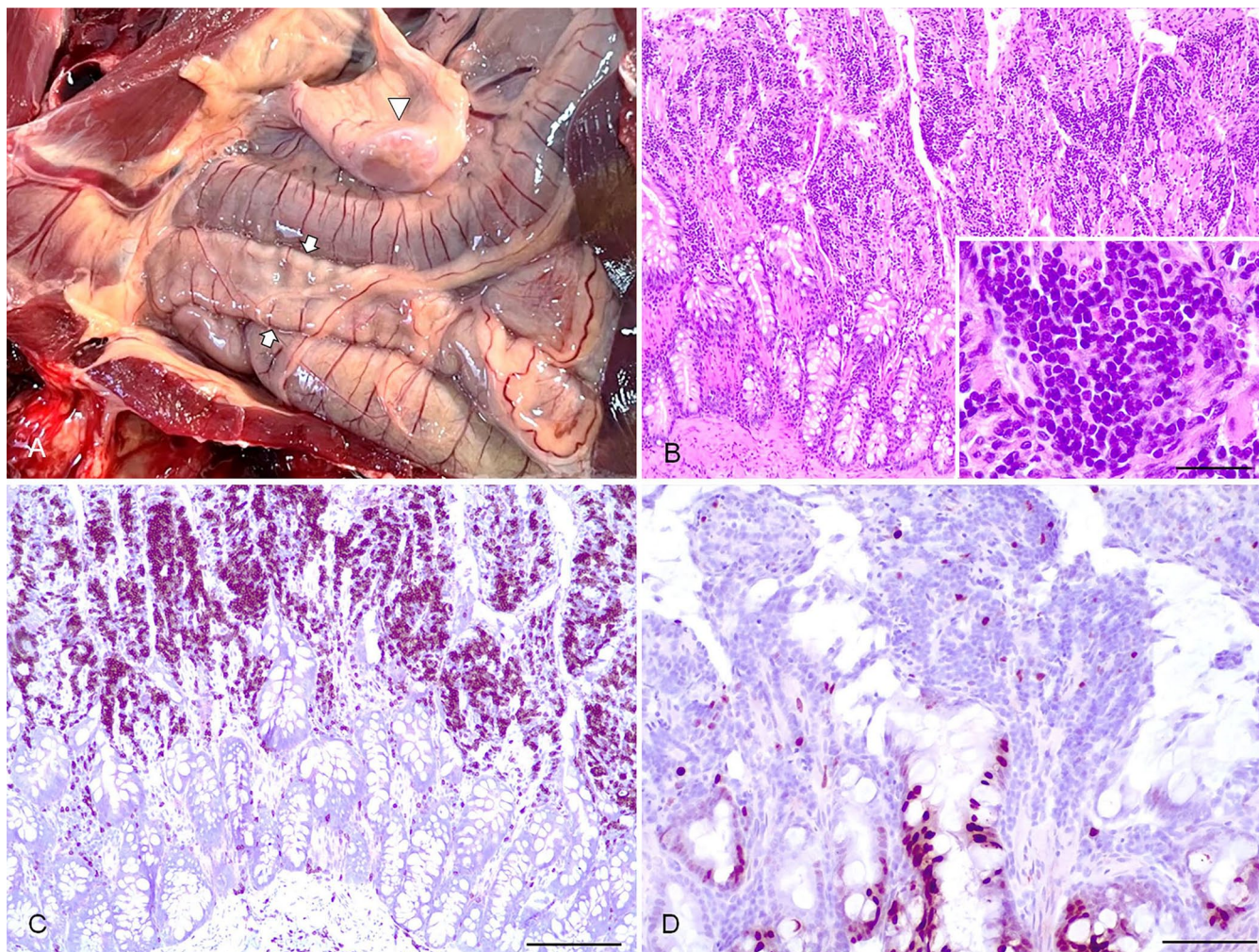
Diffuse membranous immunoreaction for CD3 was observed in neoplastic lymphocytes (T-cell lymphoma; Fig. 1C). However, neoplastic cells did not show immunoreaction

for CD79 $\alpha$ cy (<1%). The proliferation index, assessed through Ki-67 nuclear immunoreaction in neoplastic lymphocytes, was 16% (Fig. 1D). Our final diagnosis was intestinal T-cell lymphoma. Based on the World Health Organization (WHO) Classification of Hematopoietic Neoplasms in Domestic Animals, we classified this lymphoma as enteropathy-associated T-cell lymphoma (EATL) type 2.

There are scarce reports of intestinal lymphoma in otters. Most reported lymphomas in the subfamily *Lutrinae* arise from lymphoid organs (spleen, LN, thymus; Table 1). Splenic lymphomas have been reported in otters, alone or as a part of multicentric disease. A splenic marginal-zone B-cell lymphoma in an Asian small-clawed otter affected also LNs, liver, and kidney.<sup>11</sup> Similarly, in a Eurasian otter, LNs, thymus, and adrenal glands were involved as part of multicentric lymphoma.<sup>2</sup> Splenectomy was curative in the case of splenic lymphoma of T-cell origin in a North American river otter.<sup>6</sup> Lymphoblastic lymphoma affecting mesenteric LN, thymus, and liver was reported in a sea otter (*Enhydra lutris*).<sup>4</sup> Lymphomas in other locations have been reported on rare occasions, such as a T-cell lymphoma in the brain of a sea otter.<sup>13</sup>

Among mustelids, except for domestic ferrets,<sup>8,17</sup> alimentary lymphomas have only been reported in a fisher (*Pekania pennanti*) and a Eurasian otter.<sup>1</sup> Similar to our case, the Eurasian otter had a history of nephrolithiasis. In contrast, the worsening condition in the Eurasian otter was manifest as abdominal pain. The fisher and the Eurasian otter, and the Asian small-clawed otter presented here, had segmental thickening of the jejunum found at autopsy.

The intestinal lymphomas diagnosed in the fisher and the Eurasian otter were transmural lymphomas of granular lymphocyte type, considered a subentity of EATL type 1. Intestinal lymphomas of granular lymphocyte type are characterized by transmural jejunal infiltration of large neoplastic lymphocytes, sometimes with concurrent intra-tumoral eosinophils. EATL type 1 is an aggressive neoplasm that led to intestinal perforation in the Eurasian otter.<sup>1</sup>



**Figure 1.** Intestinal T-cell lymphoma in an Asian small-clawed otter. **A.** The jejunal wall is thickened irregularly, serosal lymphatic vessels are dilated (arrows), and a mesenteric lymph node is enlarged (arrowhead). **B.** Neoplastic lymphocytes are arranged in a band that expands the jejunal villus-crypt junction, and infiltrates the villi and crypts. H&E. Inset: neoplastic lymphocytes have small, round, centrally placed, 6–8- $\mu$ m nuclei, and scant, lightly eosinophilic cytoplasm. H&E. Bar=50  $\mu$ m. **C.** Neoplastic lymphocytes diffusely express CD3 (T lymphocytes). Rabbit polyclonal anti-CD3 antibody. Bar=400  $\mu$ m. **D.** The proliferation index is 16%. Rabbit monoclonal anti-Ki-67 antibody. Bar=200  $\mu$ m.

Our case of intestinal lymphoma was classified as EATL type 2 based on histologic and immunophenotypic characteristics. EATL type 2 is the most common intestinal lymphoma subtype in domestic cats,<sup>7</sup> and is the predominant type among intestinal lymphomas in ferrets.<sup>17</sup> EATL type 2 in cats is characterized by various patterns of mucosal expansion of a monomorphic population of small-to-intermediate neoplastic lymphocytes with variable degrees of epitheliotropism.<sup>9</sup> The indolent, slowly progressing course of EATL type 2 may explain the lack of specific clinical signs in our case, given that most clinical signs could be attributed to uremia.

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The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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