Supplementary Material

Immunohistochemical analysis (IHC) was performed on formalin-fixed, paraffin-embedded tissue sections via a panel of primary antibodies directed against thyroglobulin and TTF-1 (Table S1). These antigens were selected on the basis of their diagnostic value in endocrine and thyroid disorders (3, 30).

Antigen retrieval was performed via heat-induced epitope retrieval (HIER) protocols tailored to each antibody. Buffers such as EDTA (pH 8.0) or Tris-EDTA (pH 9.0) were used, with incubation at temperatures ranging from 95 to 110 °C for 15 to 40 minutes, depending on the antibody. Detection was performed via a polymeric peroxidase (HRP)-conjugated system, and immunostaining was visualized via the use of 3,3'-diaminobenzidine (DAB**)** as a chromogen (DAB substrate kit, Sigma–Aldrich, Ref. 957D). The sections were subsequently counterstained with hematoxylin. Appropriate positive controls (human and cat thyroglobulin and TTF-1) were included in each run.

Immunostaining was assessed semiquantitatively via the following scale: 0 = no staining; 1 = 5–20% positive cells; 2 = 21–50%; and 3 = more than 50% positive cells (Table S1).

Table S1. Primary antibodies and immunohistochemical conditions used for immunohistochemistry (IHC). All the antibodies were validated for use in feline tissue via appropriate positive controls. Heat-induced epitope retrieval (HIER) and polymer-based detection systems were used.

Antibody	Clone/Type	Supplier/Ref.	Dilution/Presentation	Antigenic recovery	Chromogen	Positive control	Location
Thyroglobulin	2H11+6E1, monoclonal IgG1 (mouse)	Zeta Corporation/Z2062MP	1:100–1:200 (concentrated) or prediluted	Tris-EDTA pH 9.0, 100 °C, 40 min	DAB, Sigma–Aldrich (Ref. 957D)	Thyroid – human & feline	Cytoplasmic
TTF-1	8G7G3/1, monoclonal IgG1 (mouse)	Cell Marque/Ref. 343 M-xx	1:100–1:500 (concentrated) or prediluted	Trilogy™, 110 °C, 15 min (pressure cooker)	DAB, Sigma–Aldrich (Ref. 957D)	Thyroid – human & feline	Nuclear

Immunostaining of thyroid tissue from the cats revealed a significant decrease in the expression of both thyroglobulin and TTF-1 compared with that in normal feline tissue and human control tissue. This absence of immunoreactivity suggests severe functional impairment of the gland, which is consistent with clinical hypothyroidism. Decreased or absent expression of these specific thyroid

markers could indicate severe follicular atrophy, chronic inflammation, or cellular transformation with loss of differentiation, findings that support the hypothesis of profound thyroid dysfunction in the present case.