

Patient's serum as a probe for detecting pathogens in formalin-fixed, paraffin-embedded sections

Patients' sera diluted at 1:500 or 1:1,000 can be utilized as the primary antibodies in indirect immunoperoxidase staining to detect a variety of pathogens in formalin-fixed, paraffin-embedded sections. The sera of patients with bacterial and fungal infection showed a wide range of cross-reactivity against bacteria and/or fungi, but those with protozoan and helminthic infections exhibited a good specificity to the pathogen. Almost no immunoreactivity of endogenous human IgG in the paraffin sections was demonstrated under the conditions of this study. To protect biohazard, the sera with positivity for hepatitis viruses or HIV should not be used as a probe. This approach can be used in diagnostic pathology, particularly when specific antisera or monoclonal antibodies are unavailable.

Ref.-1: Tsutsumi Y, et al. Use of patients' sera for immunoperoxidase demonstration of infectious agents in paraffin sections. *Acta Pathol Jpn* 1991; 41(9): 673-679. doi: 10.1111/j.1440-1827.1991.tb02791.x

Ref.-2: Tsutsumi Y. Histopathological diagnosis of infectious diseases using patients' sera. *Seminar Diagnost Pathol* 2007; 24(4): 243-252. doi: 10.1053/j.semdp.2007.07.005

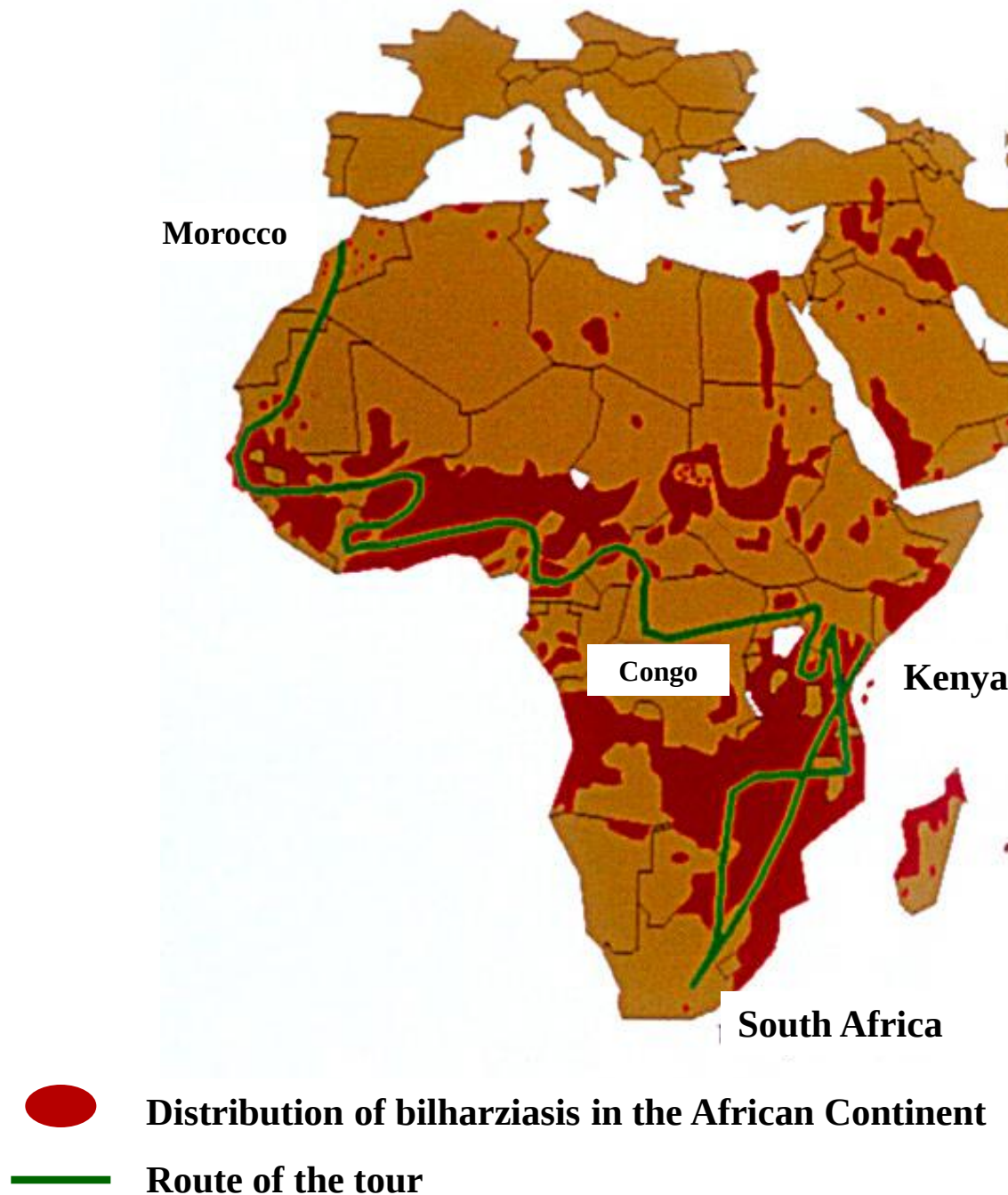
Ref.-3: Tsutsumi Y. Low-specificity and high-sensitivity immunostaining for demonstrating pathogens in formalin-fixed, paraffin-embedded sections. *IntechOpen: Immunohistochemistry. The Ageless Biotechnology* (ed. Streckfus CF). 2019; doi: 10.5772/intechopen.85055

Immunostaining using patients serum: Examples

- 1) Staphylococcal pyoderma
- 2) Propionibacterium acnes folliculitis
- 3) Cat scratch disease
- 4) Tsutsugamushi disease
- 5) Varicella-zoster virus infection
- 6) Cryptococcosis
- 7) Sporotrichosis
- 8) Malassezia folliculitis
- 9) Cutaneous alternariosis
- 10) Amebic dysentery
- 11) Acanthamoeba infection
- 12) Balamuthia encephalitis
- 13) Cutaneous leishmaniasis
- 14) Visceral leishmaniasis
- 15) Cryptosporidiosis
- 16) Cystoisosporiasis
- 17) Toxoplasmosis
- 18) Microsporidiosis
- 19) Blastocystis hominis infection
- 20) Ascariasis
- 21) Anisakiasis
- 22) Gnathostomiasis
- 23) Angiostrongylosis
- 24) Bilharziasis
- 25) Japanese schistosomiasis
- 26) Multilocular echinococcosis
- 27) Neurocysticercosis

Points of the techniques

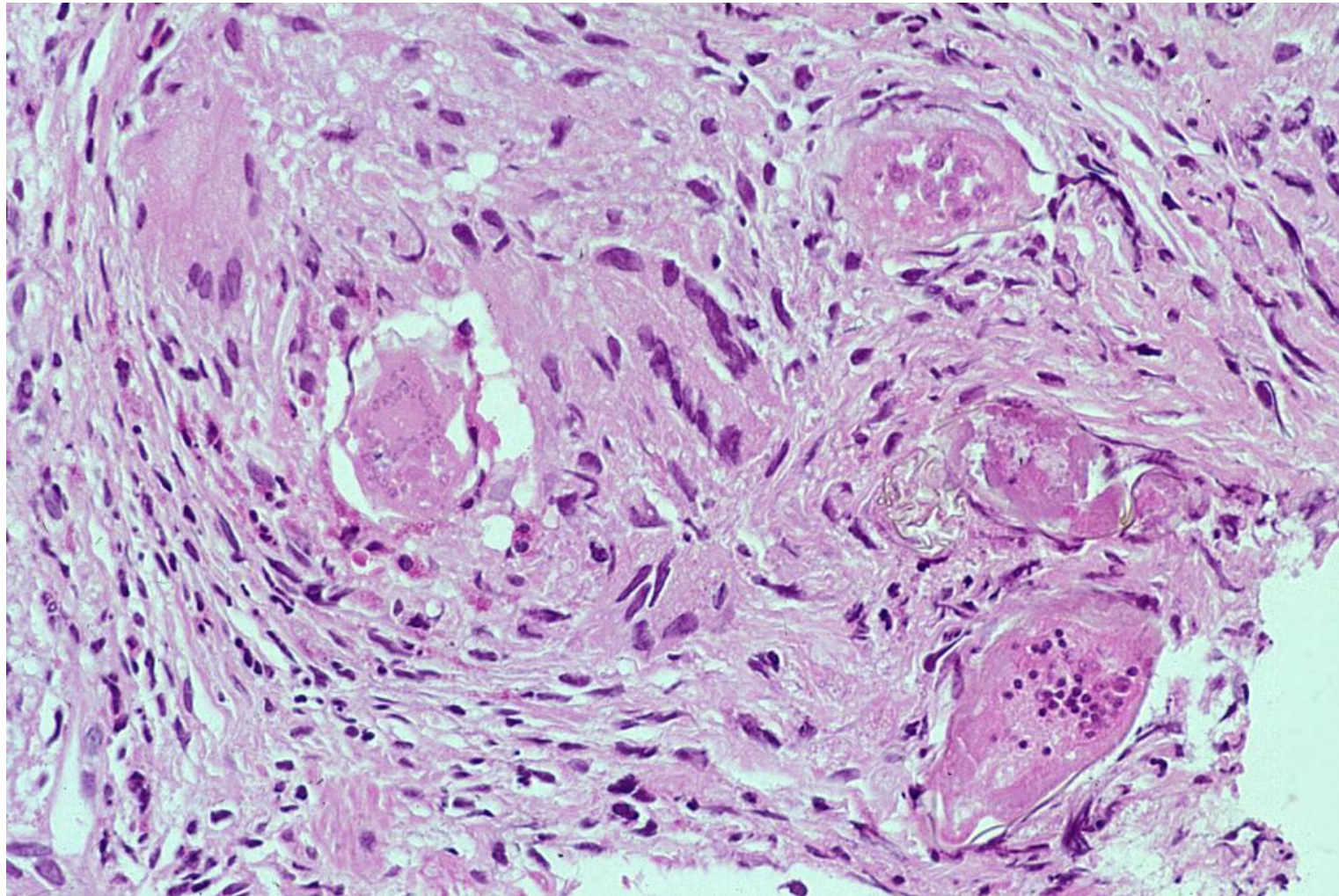
- 1) Microscopic recognition of host responses to the pathogens, such as abscess or granuloma, in histopathologic sections indicates the production of specific IgG antibodies to pathogens in question.
- 2) Confirmation of the negativity of hepatitis virus markers and HIV in the serum is important to avoid biohazard during staining.
- 3) The indirect immunoperoxidase method should be applied (the method with high sensitivity of detection should not be used to avoid the background staining by endogenous IgG in the body fluid).
- 4) The patients' sera can be used at 1:500 or 1:1,000 dilution.
- 5) The specificity of the patient's serum is high in case of protozoan or helminthic infections.
- 6) Once the specificity of the patient's serum is confirmed, it can be used as a primary specific antibody to other cases.



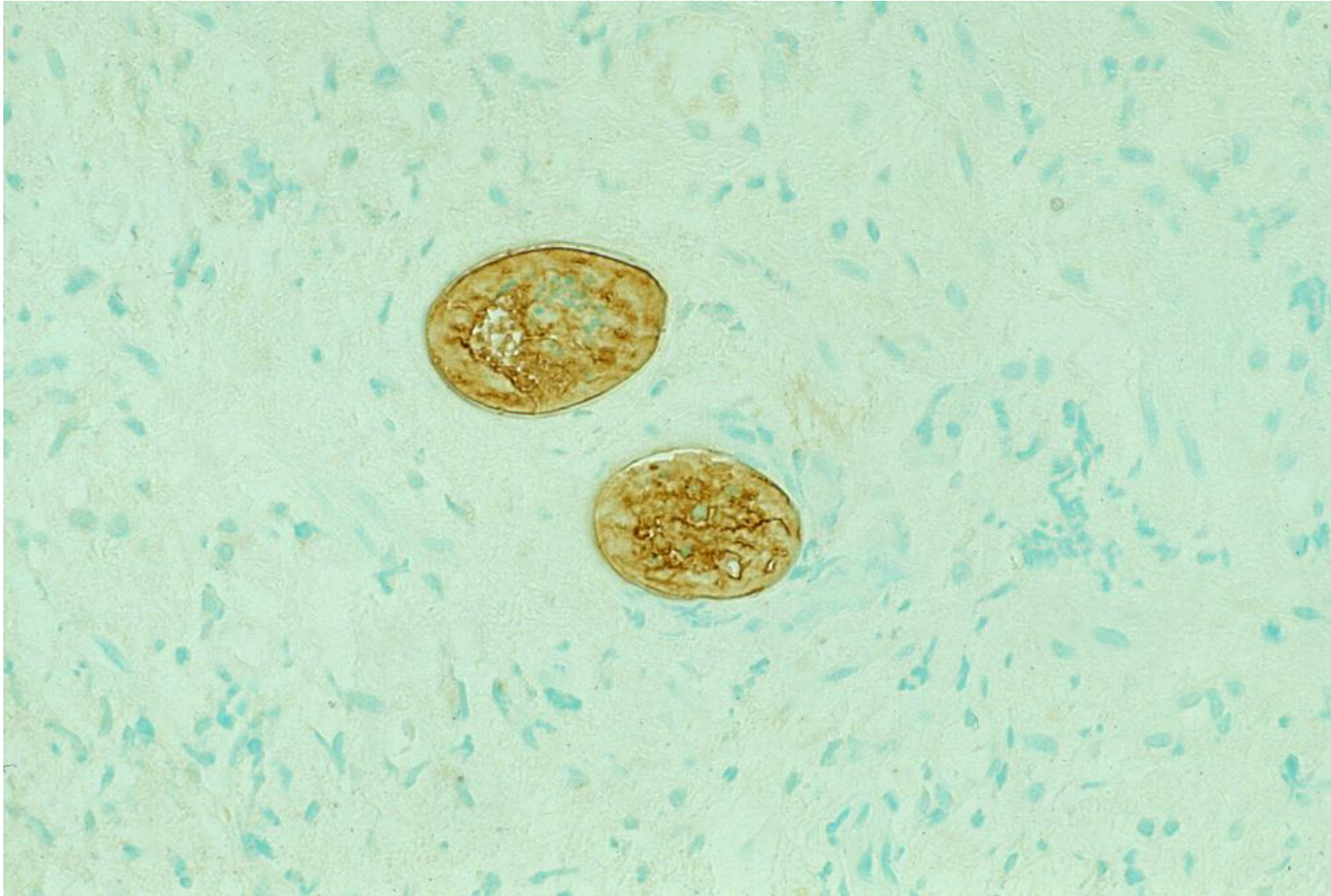
A Japanese young guy aged 20's tried the survival tour across the African Continent for 3 months without using hotels



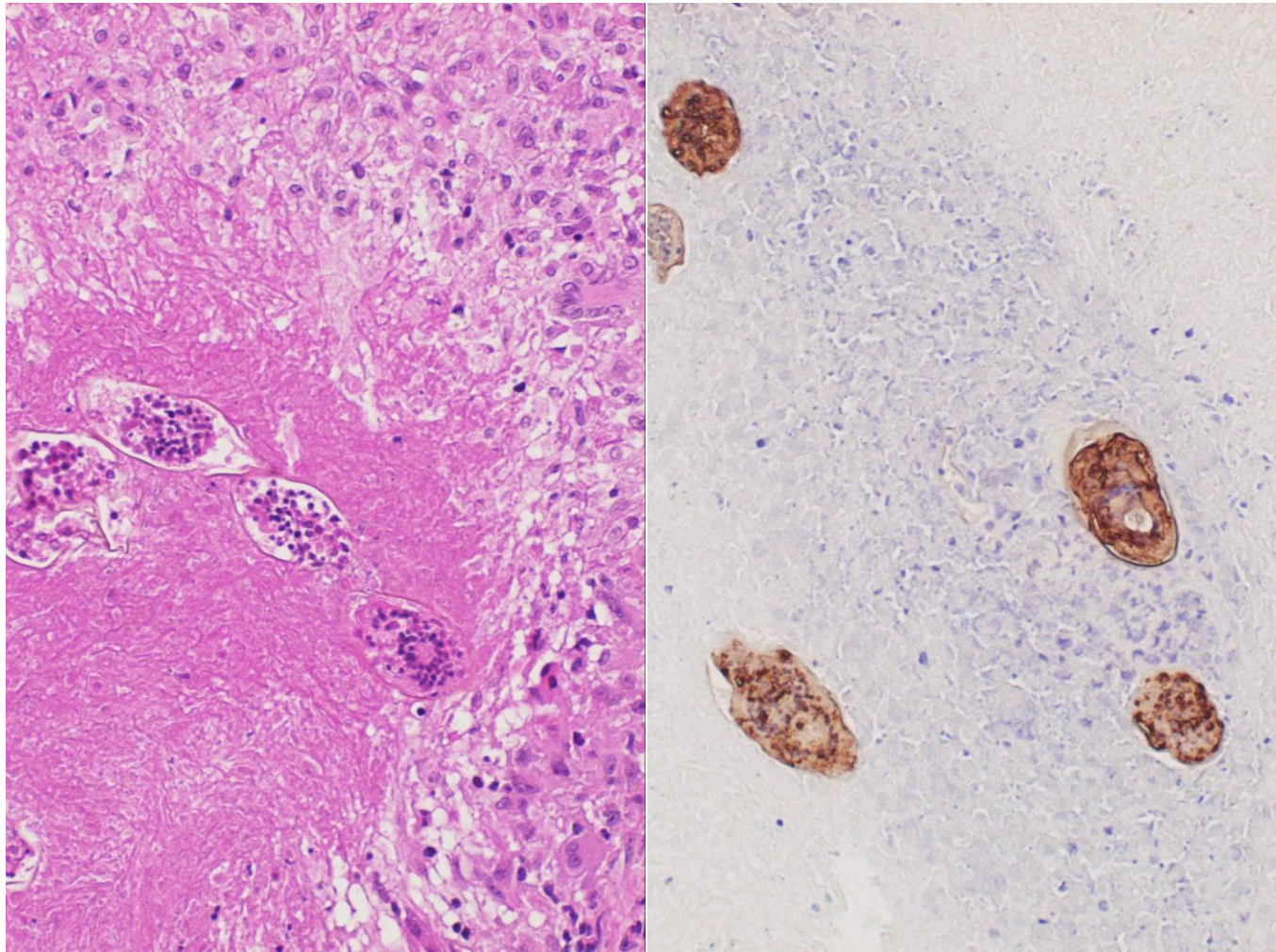
Bilharziasis. Two years after attending the survival tour, the Japanese young guy complained of hematuria without pain. The unstained urine sediment showed a spiked egg of *Schistosoma haematobium* containing a miracidium covered with moving cilia, in the background of red blood cells.



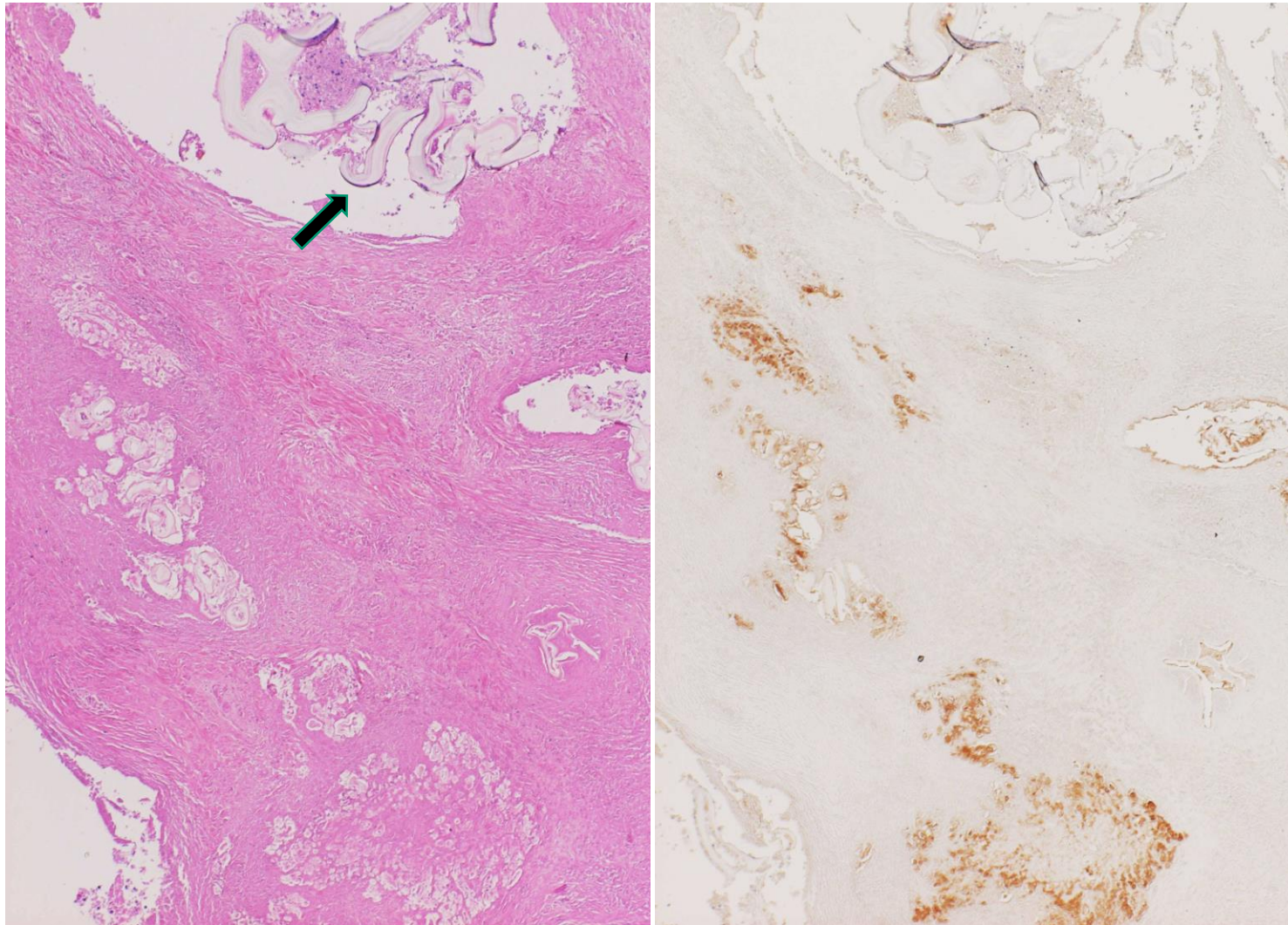
Bilharziasis. Colonofiberscopic examination disclosed a whitish spot, and biopsy revealed egg nodules. The ova with miracidium are surrounded by epithelioid granuloma. *Schistosoma haematobium* infests the pelvic veins around the urinary bladder. H&E



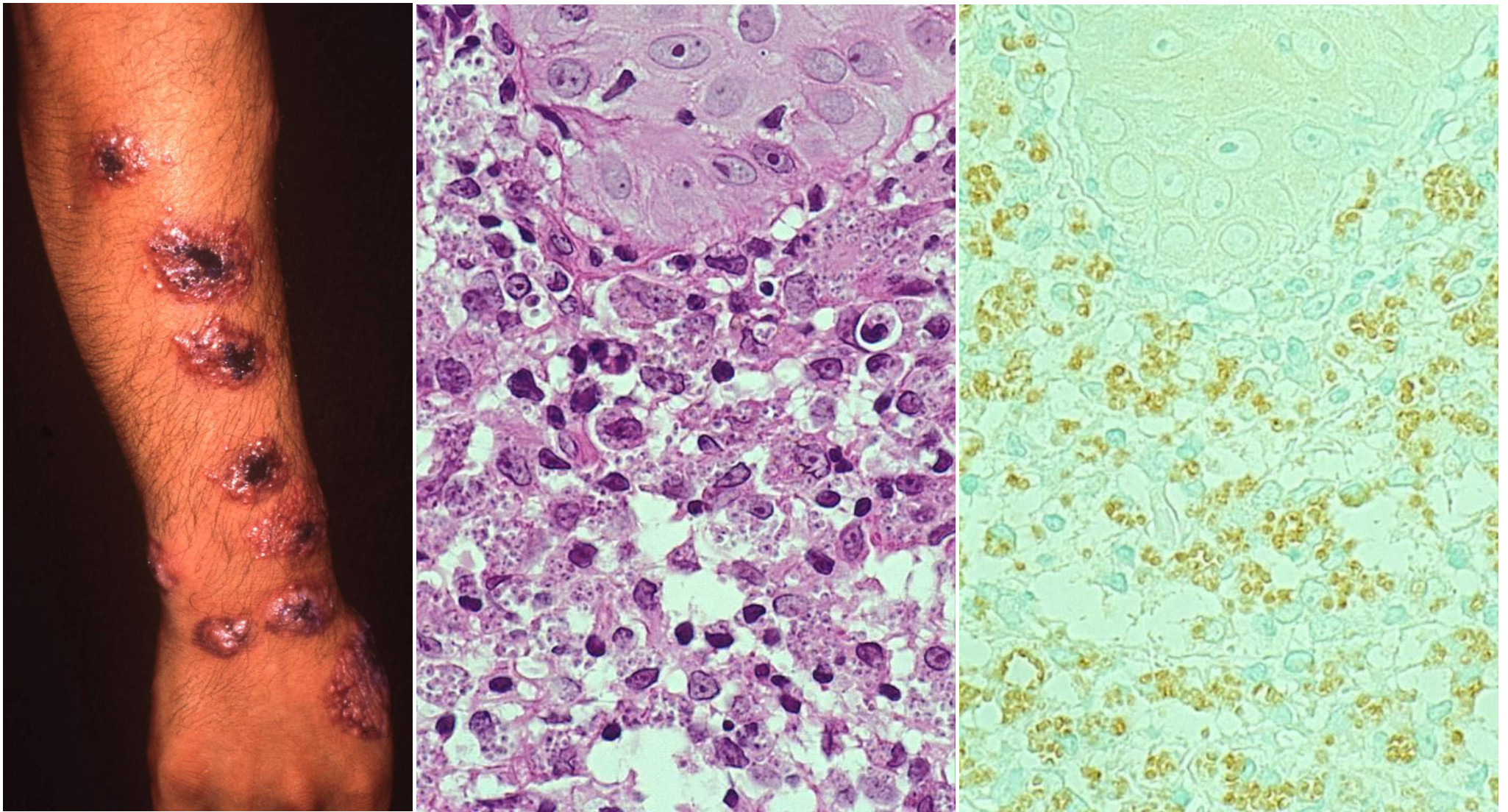
Bilharziasis. Indirect immunoperoxidase staining using the 1:500 diluted patient's own serum clearly detect the content of the eggs in the egg tubercle.



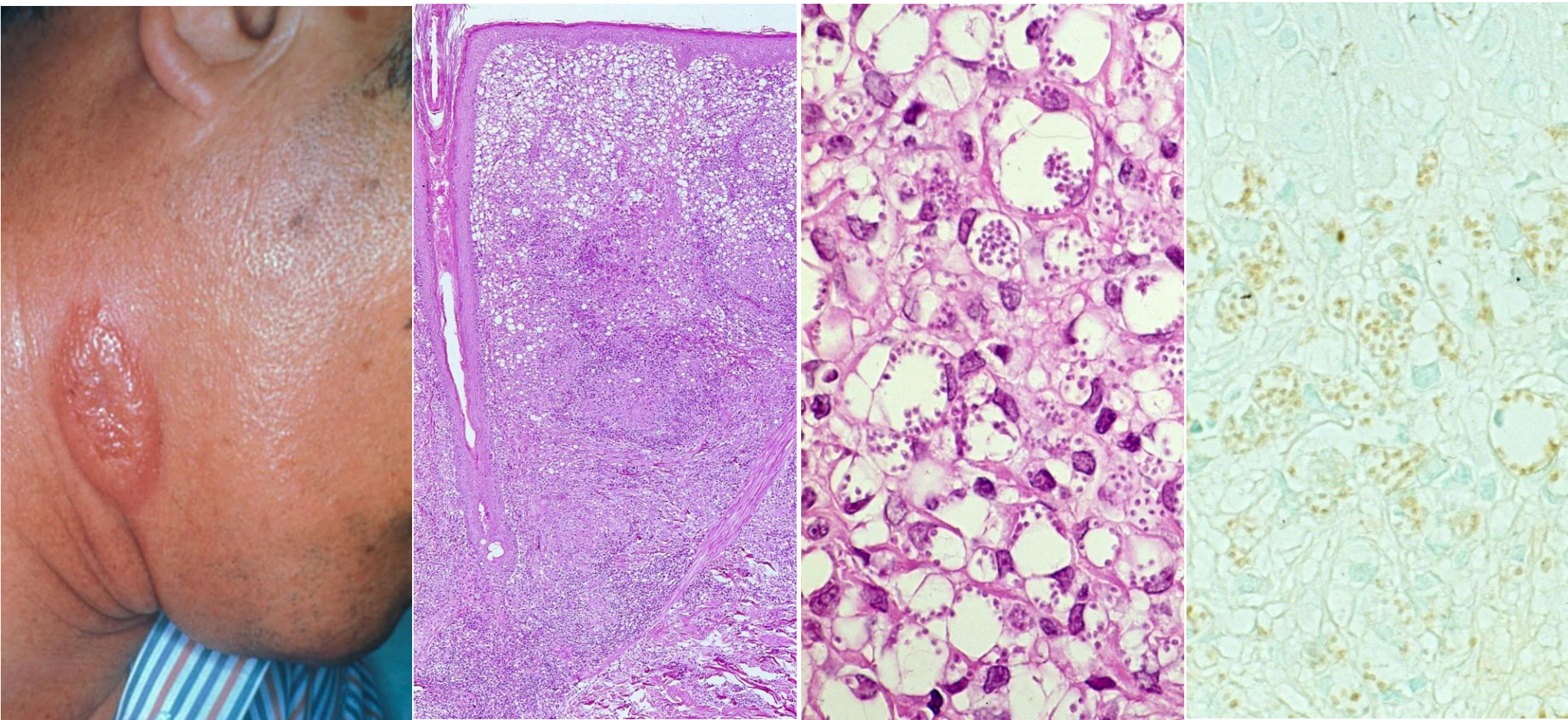
Bilharziasis of the brain; left, H&E; right, reactivity with patient's own serum. A young Japanese male patient with a history of staying in African continent complained of epileptic seizures. The ectopic egg tubercles was excised from the brain, respectively. The multinucleated (miracidial) content of the eggs of *Schistosoma haematobium* is strongly reactive with the patient's own serum.



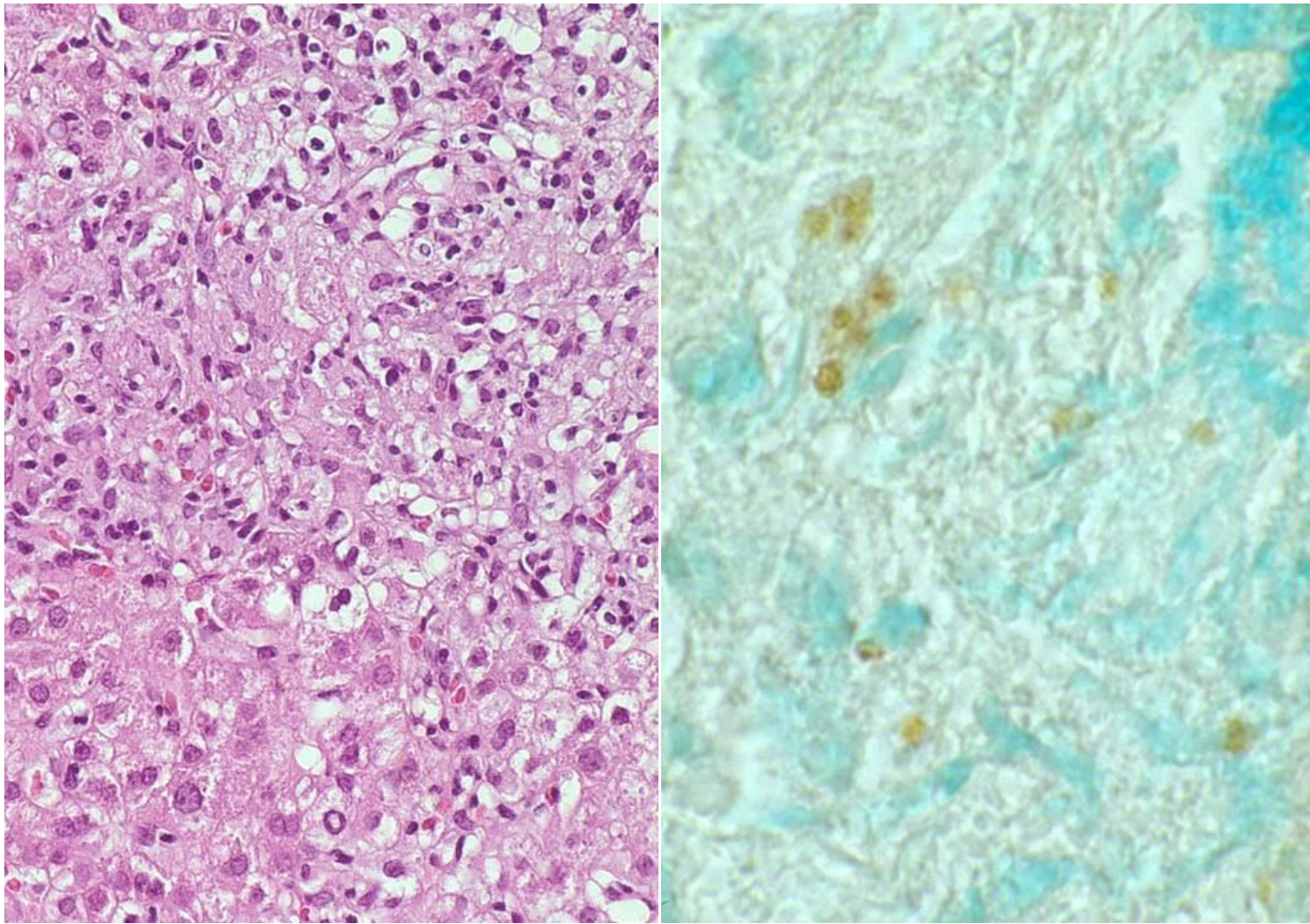
Multilocular echinococcosis. Left, H&E; right, reactivity with patient's own serum. The liver infested by *Echinococcus multilocularis* was surgically removed from a female patient living in Hokkaido. The multilocular hydatid cyst wall has been collapsed and embedded in the fibrous tissue. The diluted patient's serum is reactive with the collapsed cyst wall component, while the intact hydatid cyst wall (arrow) reveals negativity.



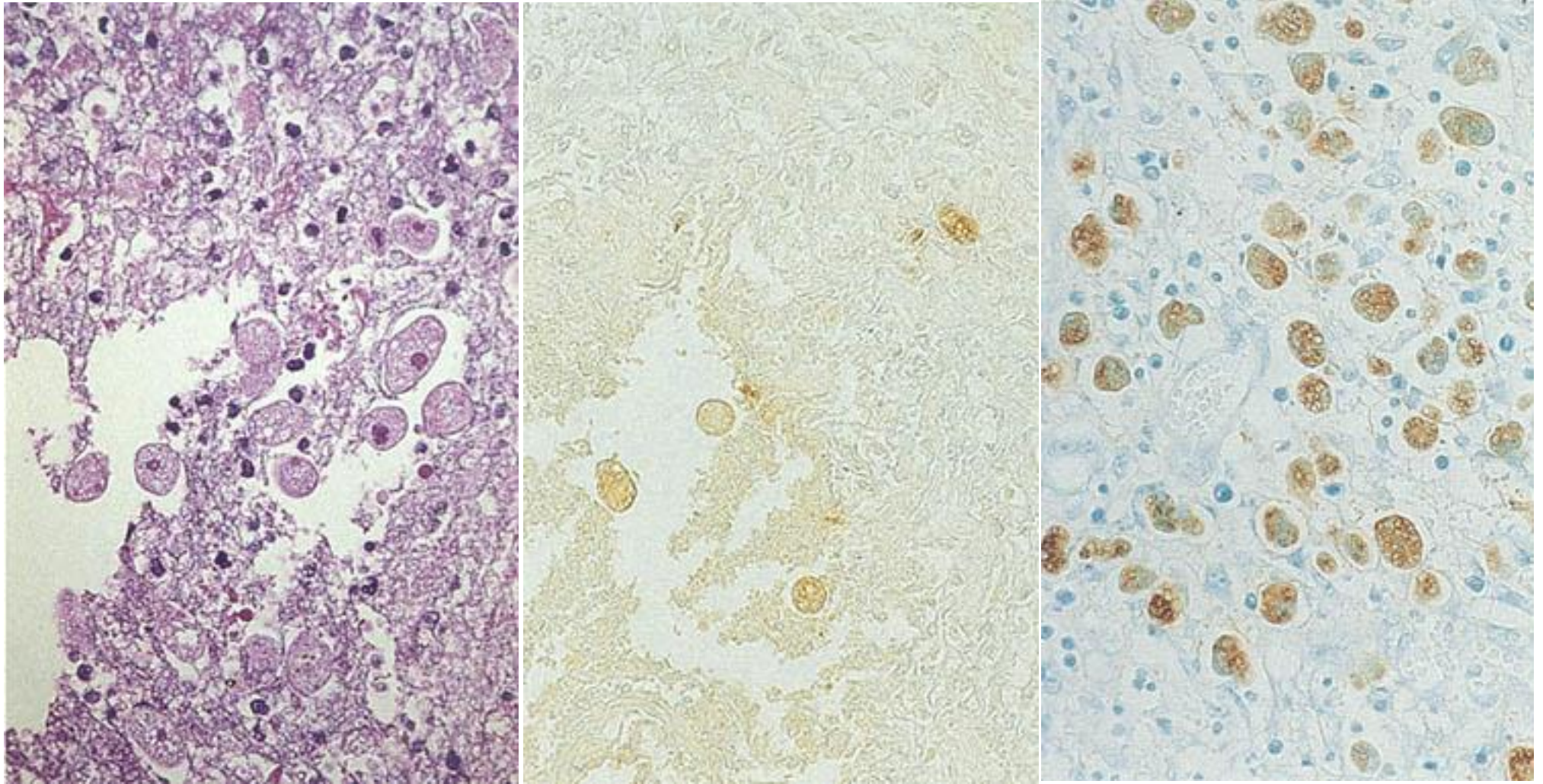
Cutaneous leishmaniasis of African type (*L. major* infestation). This Japanese guy infected *L. major* during volunteer plantation in the desert in Mali, Africa. Five months later, multiple skin ulcers were formed at the site of sand fly (*Phlebotomus*) bite. Left: gross appearance of the arm, center: H&E, right: indirect immunoperoxidase staining using 1:500 diluted patient serum.



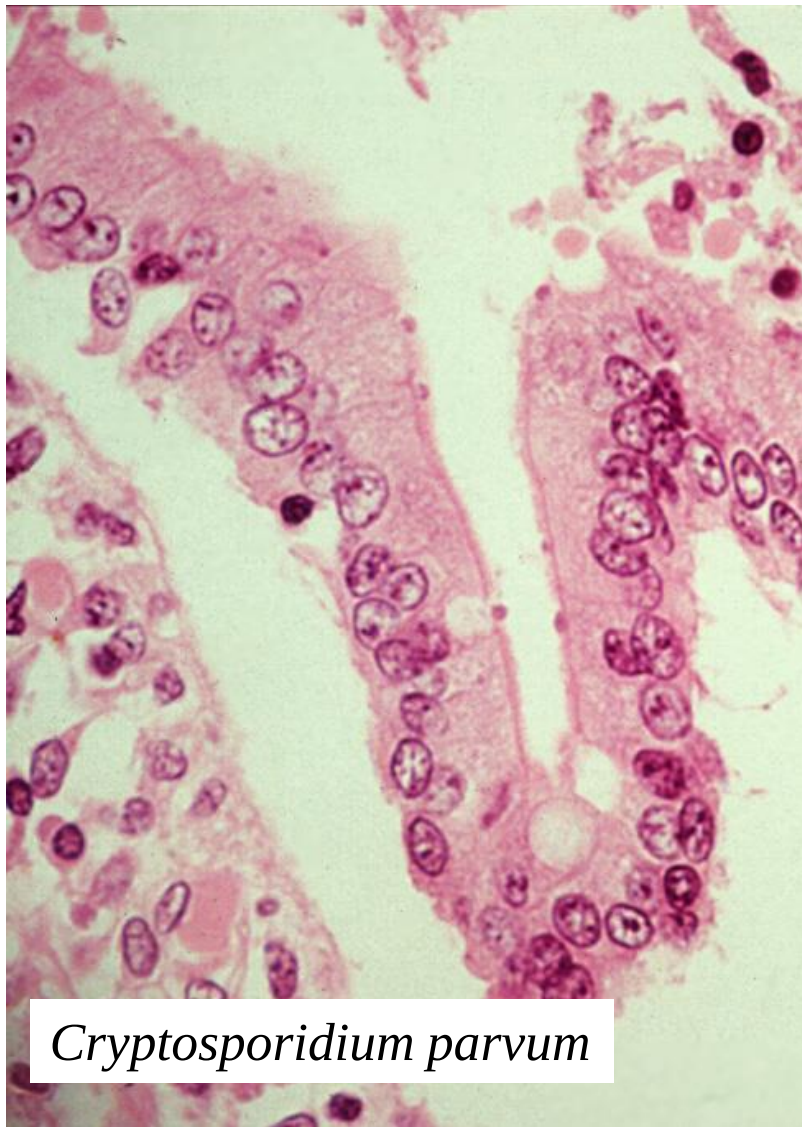
Cutaneous leishmaniasis of Indian type (*L. tropica* infestation). This Japanese businessman aged 60's infected *L. tropica* during the stay in India. Four months later, a single skin ulcer was formed on the neck skin. Left: gross appearance of the neck, center (two panels): H&E, right: indirect immunoperoxidase staining using 1:500 diluted patient serum. Note that the patient's serum is not cross-reactive with *L. major* shown in the above slide.



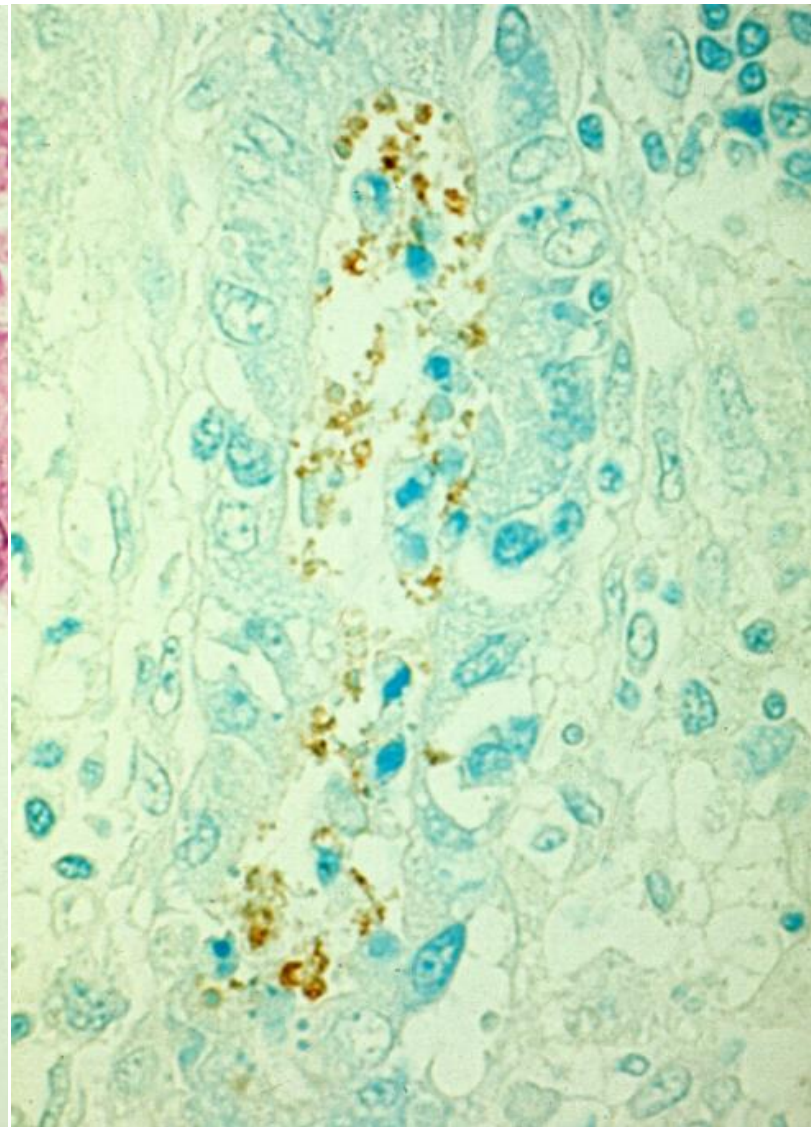
Liver biopsy of **visceral leishmaniasis** (Kala azar). A Japanese businessman worked in India manifested persistent high fever, liver dysfunction and disseminated intravascular coagulopathy. Liver biopsy discloses non-caseous microgranulomas. Since immunostaining with the antibody panel against pathogens failed to detect pathogens, the immunostaining using the 1:1,000 diluted patient's own serum was performed. Red cell-sized pathogens were identified in the granulomatous lesion, and protozoan infection was strongly suspected. *Leishmania donovani* infection was then serologically confirmed.



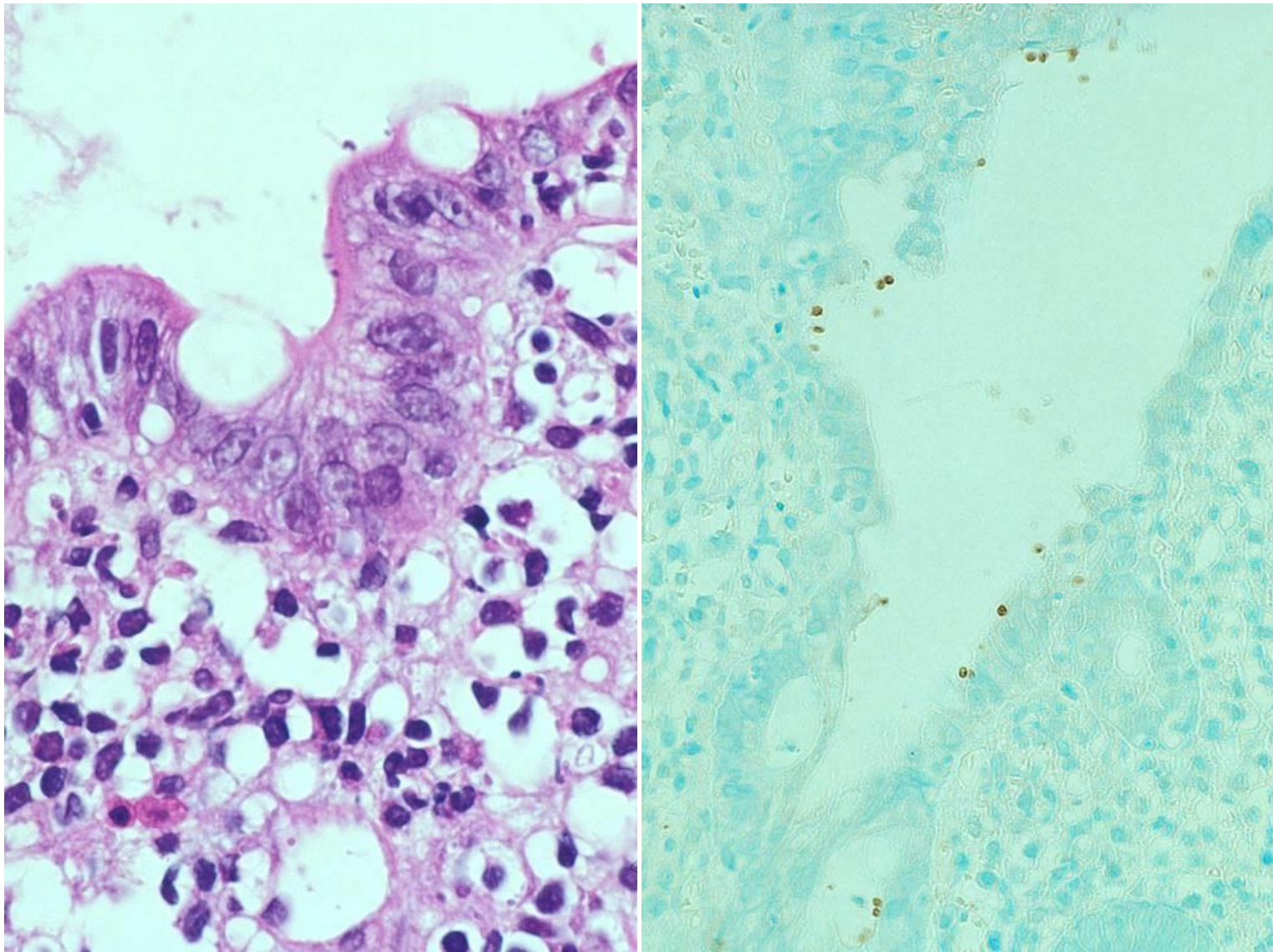
A surgical specimen of **amebic dysentery** seen in an HIV-negative male patient. Amebic trophozoites are clustered in the ulcerated base (left: H&E). The trophozoites are positively labeled with the 1:500 diluted patient's own serum (center). The pathogens are also visualized with immunostaining using a monoclonal antibody to *Entamoeba histolytica* (right).



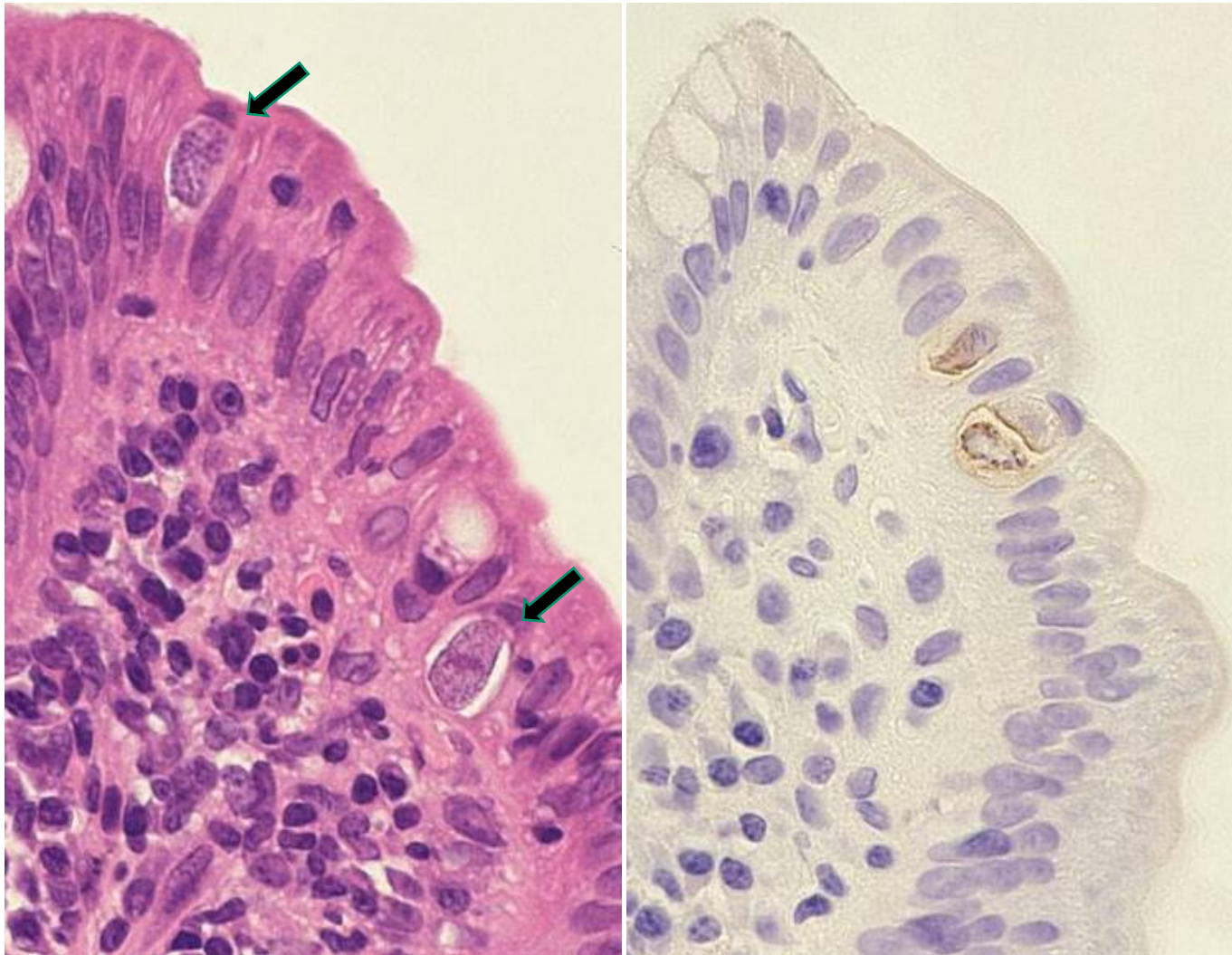
Cryptosporidium parvum



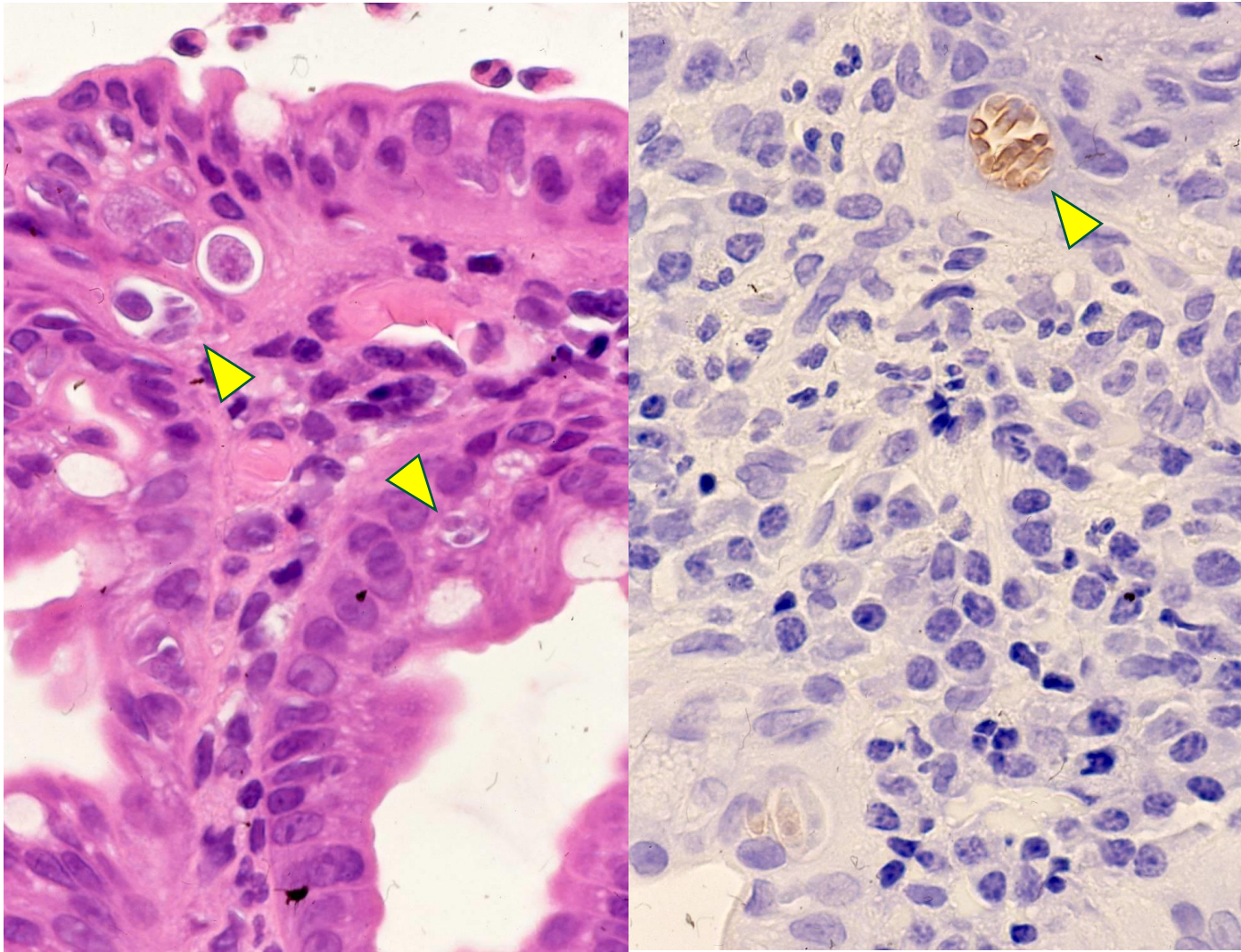
Cryptosporidiosis of the small bowel seen in a patient with AIDS. Intractable diarrhea killed the patient. Small protozoan particles are seen in the brush border of the intestinal epithelial cells. The pathogens are visualized with the indirect immunoperoxidase staining using 1:1,000 diluted serum of another HIV-negative patient suffering from acute cryptosporidial infection in an outbreak in the city. High antibody titer against *Cryptosporidium parvum* had been confirmed.



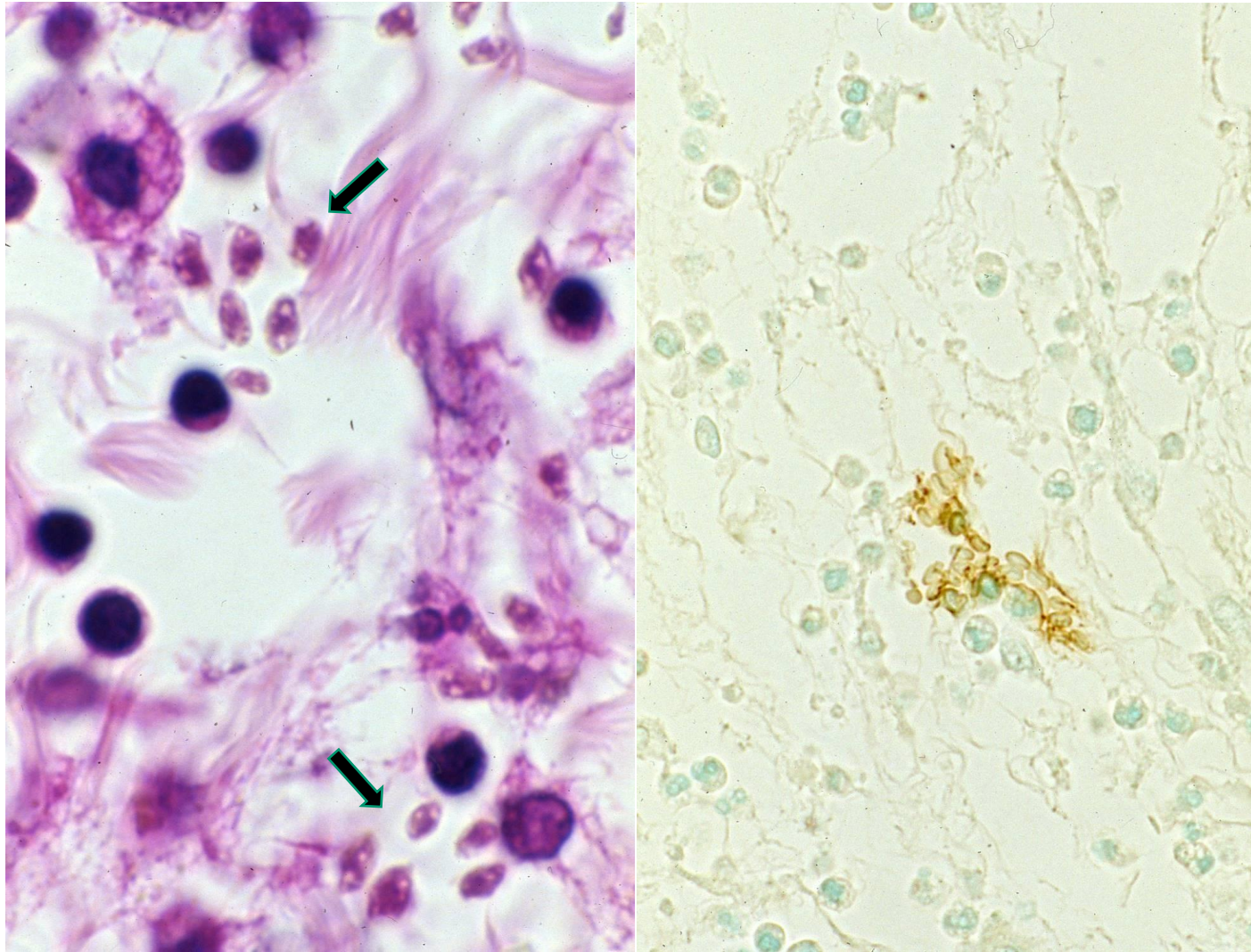
Cryptosporidiosis. Terminal ileal biopsy of acute cryptosporidiosis from a teenager boy with severe watery diarrhea. He had a close contact with cow during summer vacation in Hokkaido, a northern island, Japan. Small basophilic particles along the brush border of the epithelial cells (left: H&E) are immunoreactive with 1:500 diluted patient's own serum (right).



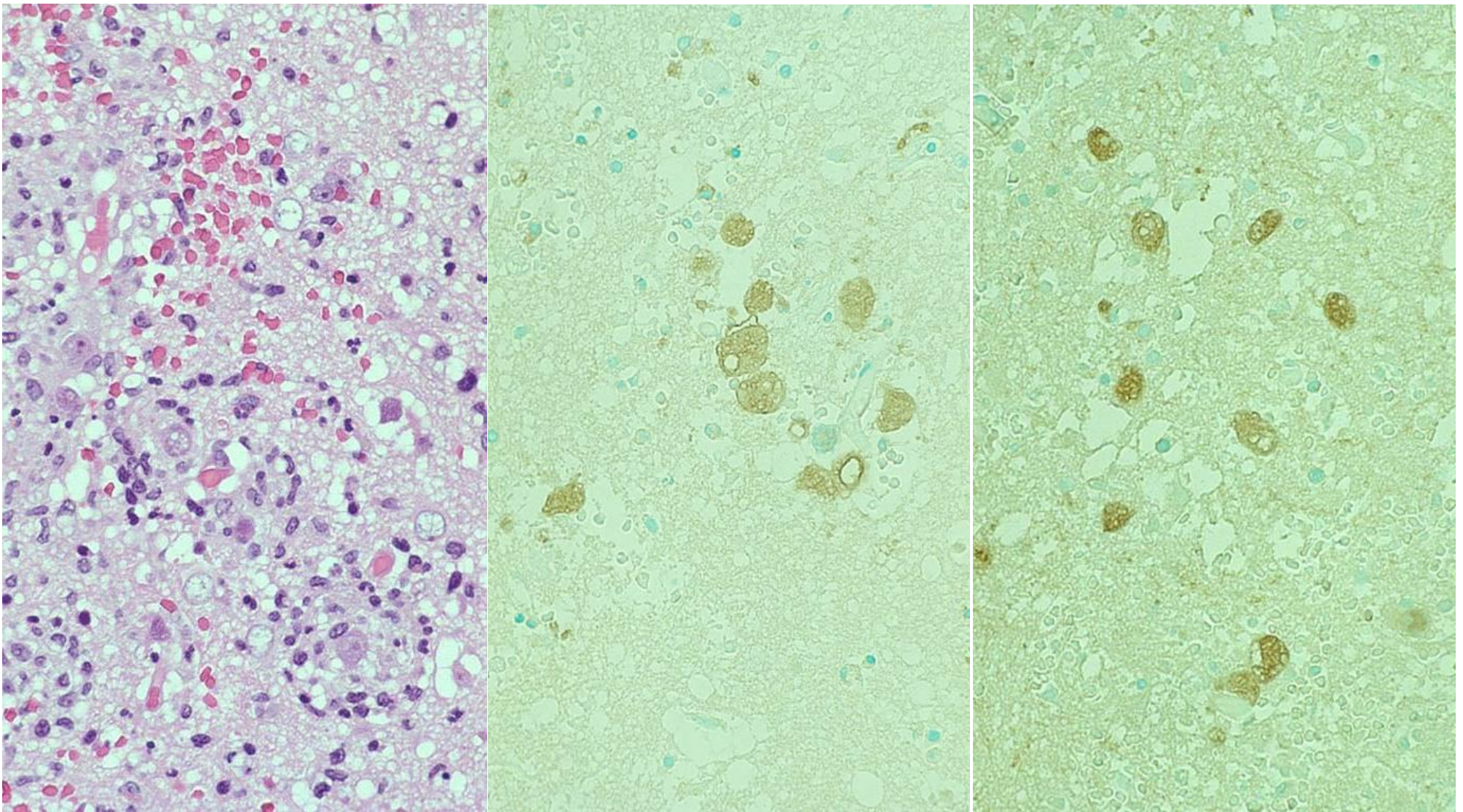
Cystoisosporiasis. Left, H&E; right, reactivity with patient's own serum. Duodenal biopsy specimen was taken from a Japanese male patient with adult T-cell leukemia in Kyushu Island, complaining of severe diarrhea. Large-sized oocysts (macrogamete) of *Cystoisospora belli* are scattered among the columnar cells (arrows). Immunostaining using patient's own serum decorates the microbe.



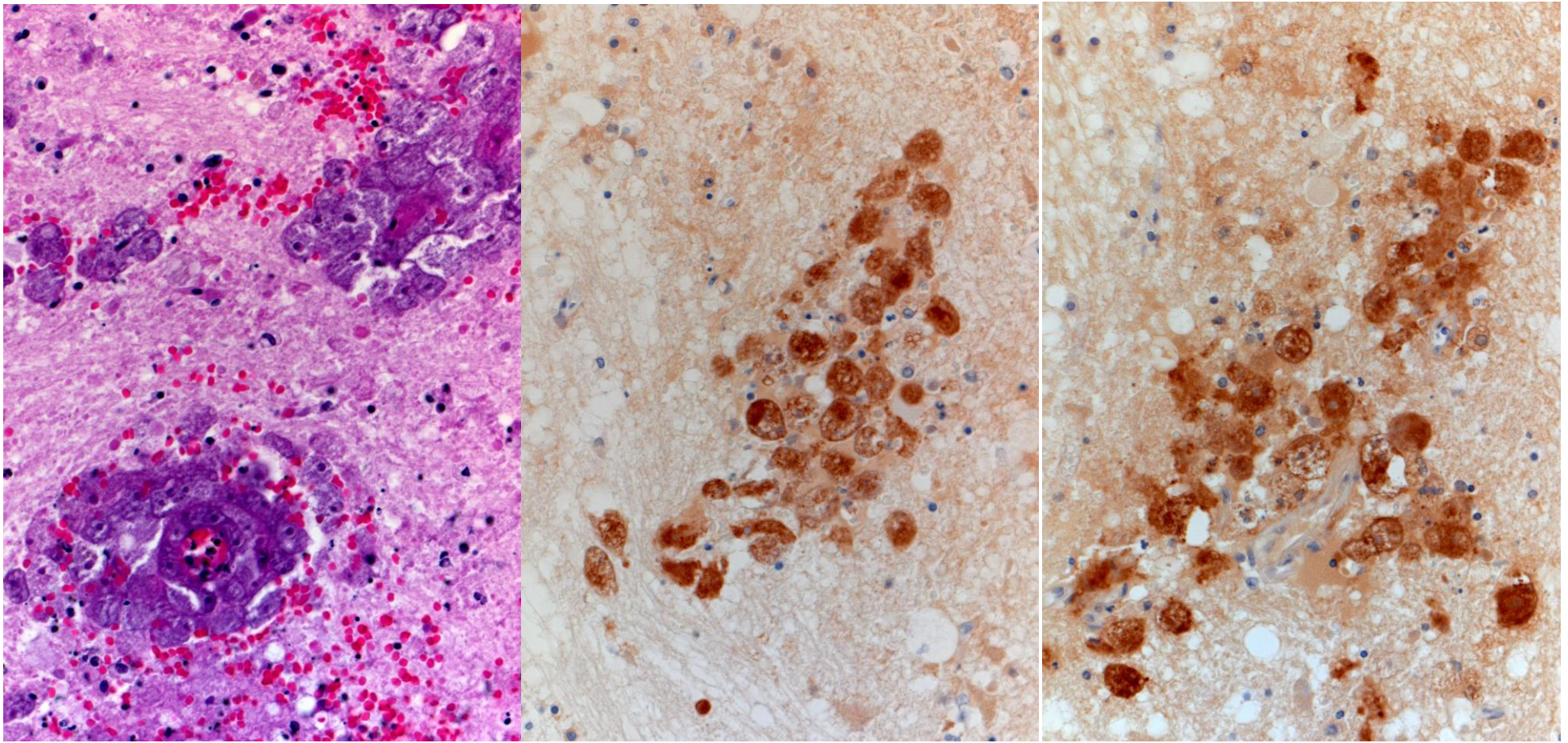
Cystoisosporiasis. Left, H&E; right, reactivity with patient's own serum. Duodenal biopsy specimen was taken from a Japanese male patient with adult T-cell leukemia in Kyushu Island, complaining of severe diarrhea. Banana-shaped merozoites of *Cystoisospora belli* are focally clustered among the columnar cells (arrowheads). Immunostaining using patient's own serum decorates the merozoites.



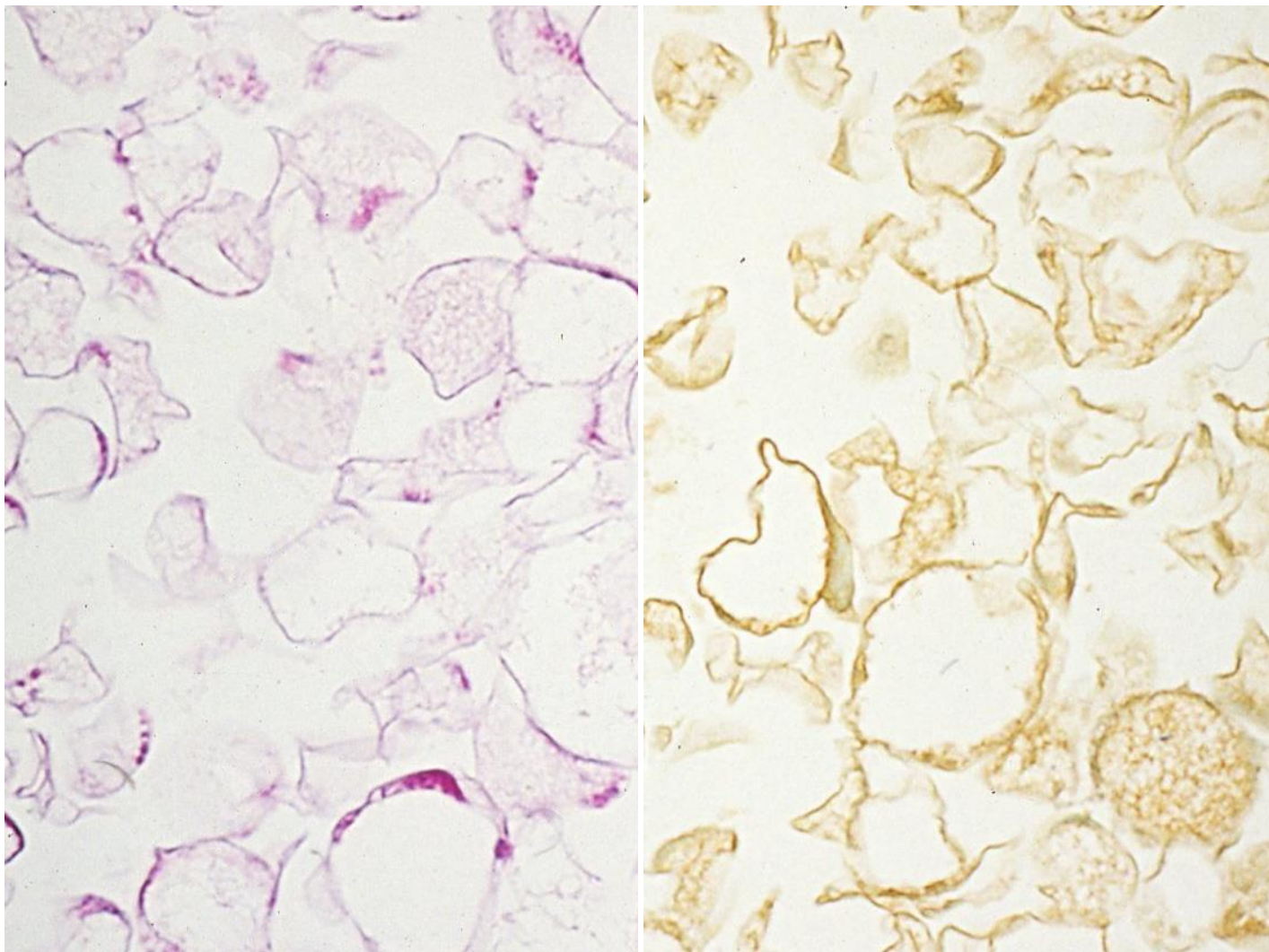
Toxoplasma meningitis in a non-AIDS patient. Left, H&E; right, reactivity with the diluted serum from a healthy individual serologically with high-titer IgG. Trophozoites of *Toxoplasma gondii* (arrows) are dispersed in the meningeal space in an aged HIV-negative female with progressive supranuclear palsy. The patient's serum decorates the pathogens.



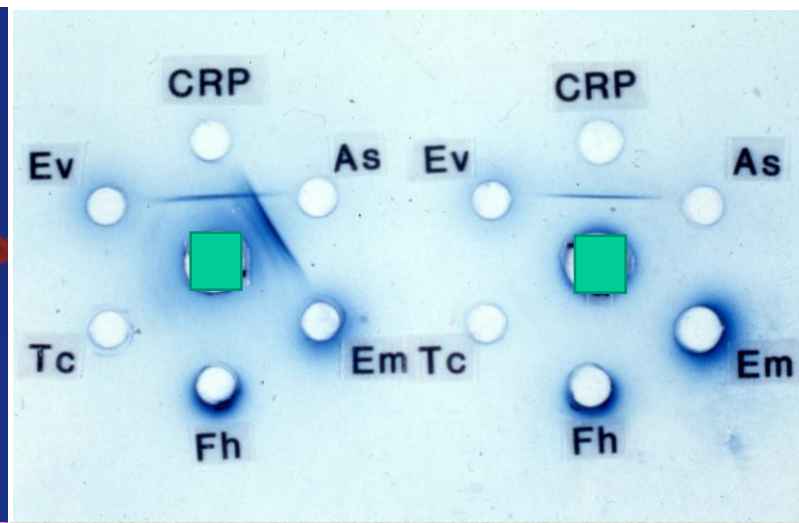
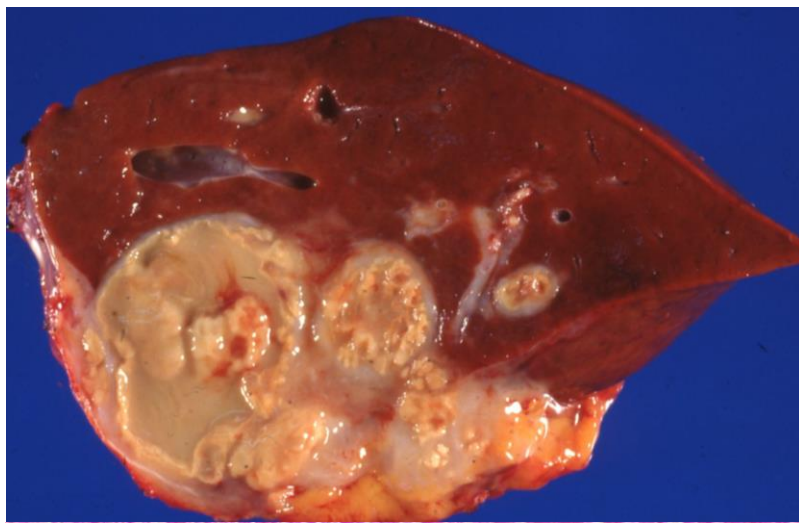
Brain biopsy of **acanthamoebic encephalitis**. The patient suffered from alcoholic liver cirrhosis. Protozoan trophozoites are scattered in the granulomatous brain lesion (left: H&E). The 1:1,000 diluted patient's own serum clearly detected the pathogens (center). A mouse antiserum against *Acanthamoeba castellanii* is also reactive (right). **Ref.:** Tsutsumi Y, et al. Acanthamebic meningoencephalitis associated with alcoholic liver cirrhosis. Pathol Case Rev 2002; 7(6):273-277. doi: 10.1097/00132583-200211000-00006



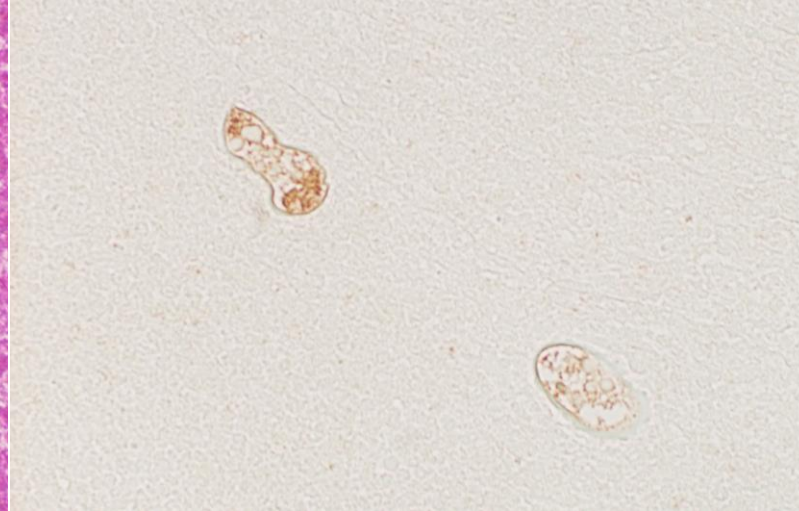
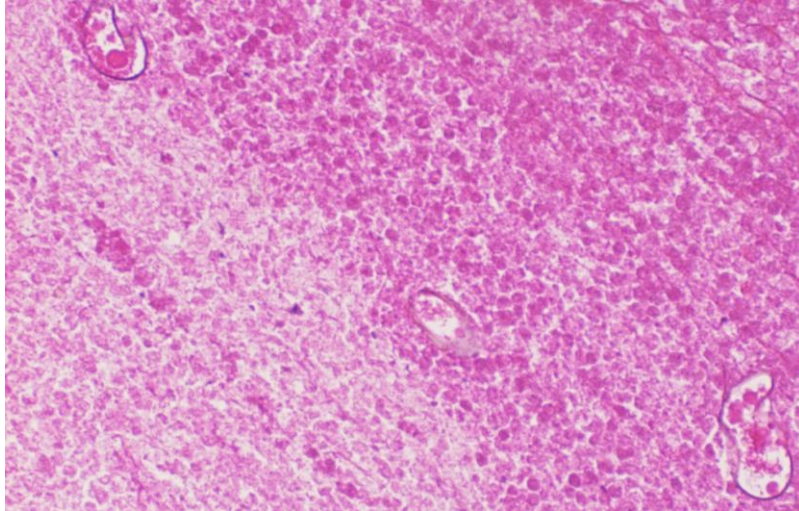
Balamuthia meningoencephalitis (left, H&E; center, reactivity with patient's own serum; right, reactivity with the serum of patient of acanthamoebic meningoencephalitis). A healthy Japanese farmer housewife complained of progressive consciousness disturbance and seizure. At autopsy, hemorrhagic meningoencephalitis was observed. Large-sized, basophilic amebic trophozoites are microscopically clustered in Virchow-Robin's spaces. PCR analysis has disclosed infection of *Balamuthia mandrillaris*. Immunostaining using both the patient's own serum and the patient's serum of the abovementioned acanthamoebic meningoencephalitis gives distinct positivity. Of note is that *Acanthamoeba* antiserum is cross-reactive with *Balamuthia*, and that heat-induced antigen retrieval is needed for visualizing *B. mandrillaris* antigens.



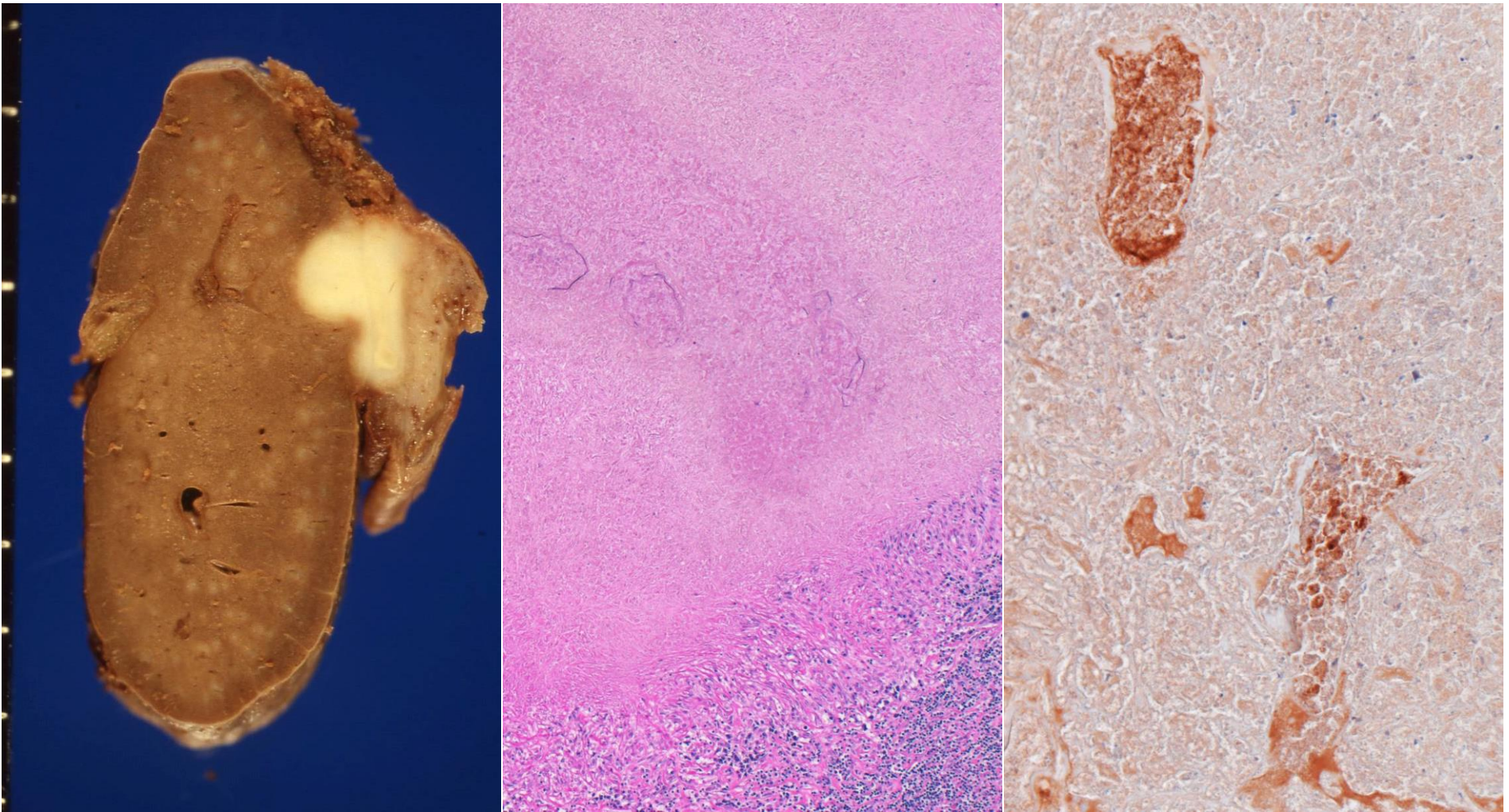
Blastocystosis. Cultured *Blastocystis hominis* in paraffin-embedded cell block preparation: left, H&E; right, reactivity with the serum of a patient with serologically proven high-titer IgG. Cultured *B. hominis* reveals vacuolated morphology and size variation. The cell wall of the microbes is immunostained with the serum of a patient with diarrhea and high-titer serum antibody to *B. hominis*.
Ref.: Kaneda Y, et al. Serologic response to *Blastocystis hominis* infection in asymptomatic individuals. Tokai J Exp Clin Med 2000; 25(2): 51-56. PMID: 11127507



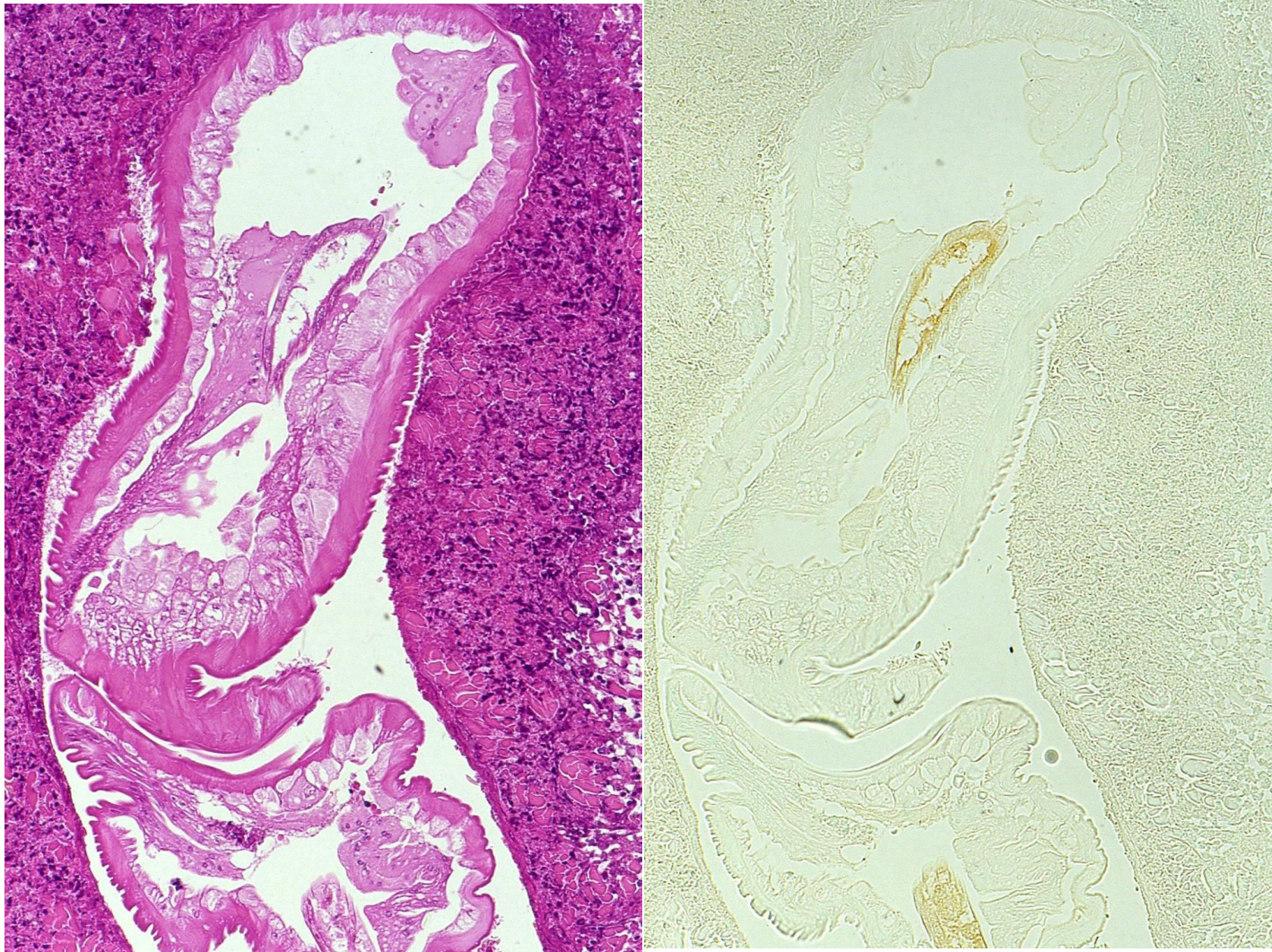
As: *Ascaris lumbricoides*
 Em: *Echinococcus multilocularis*
 Fh: *Fasciola hepatica*
 Tc: *Toxocara canis*
 EV: *Enterobius vermicularis*
 CRP: C-reactive protein



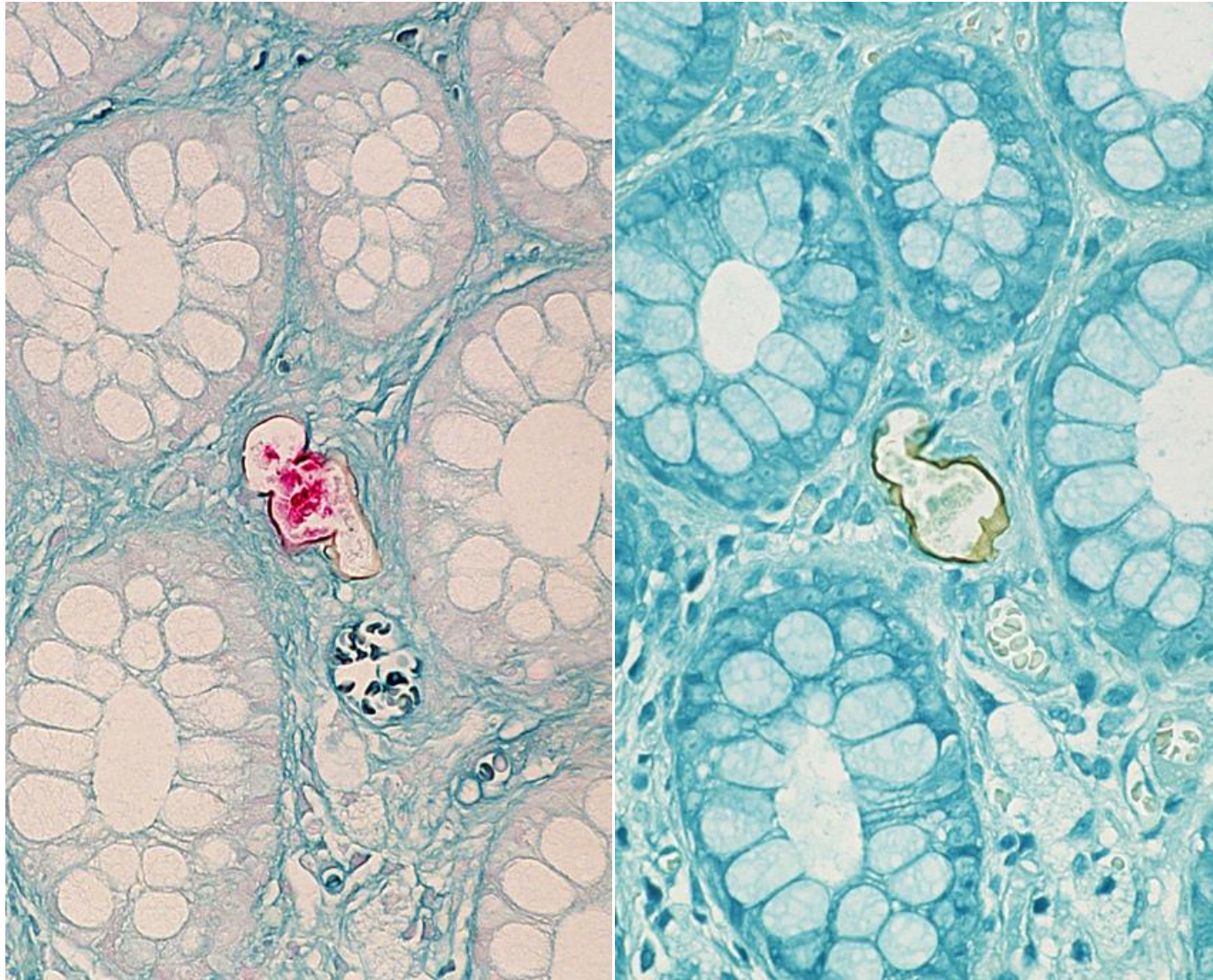
Hepatic ascariasis (left top, cut surface of the resected liver; right top, Ouchterlony's diffusion-in-gel test using the patient's serum and extract of varied helminths; left bottom, H&E; right bottom, reactivity with patient's own serum). Yellow-colored xanthogranulomatous nodules are seen at the liver hilum. Microscopically, a small number of dead and calcified ova are distributed in necrotic substance. The content of the ova is immunoreactive with the patient's serum. In Ouchterlony's test, a precipitation line against *Ascaris lumbricoides* (As) has been absorbed by the *Ascaris* extract, as shown on the right hand. **Ref.:** Nagakura K, et al. Serologic findings in hepatic ascariasis: a case report. *Tohoku J Exp Med* 1992; 167(2): 121-126. doi: 10.1620/tjem.167.121



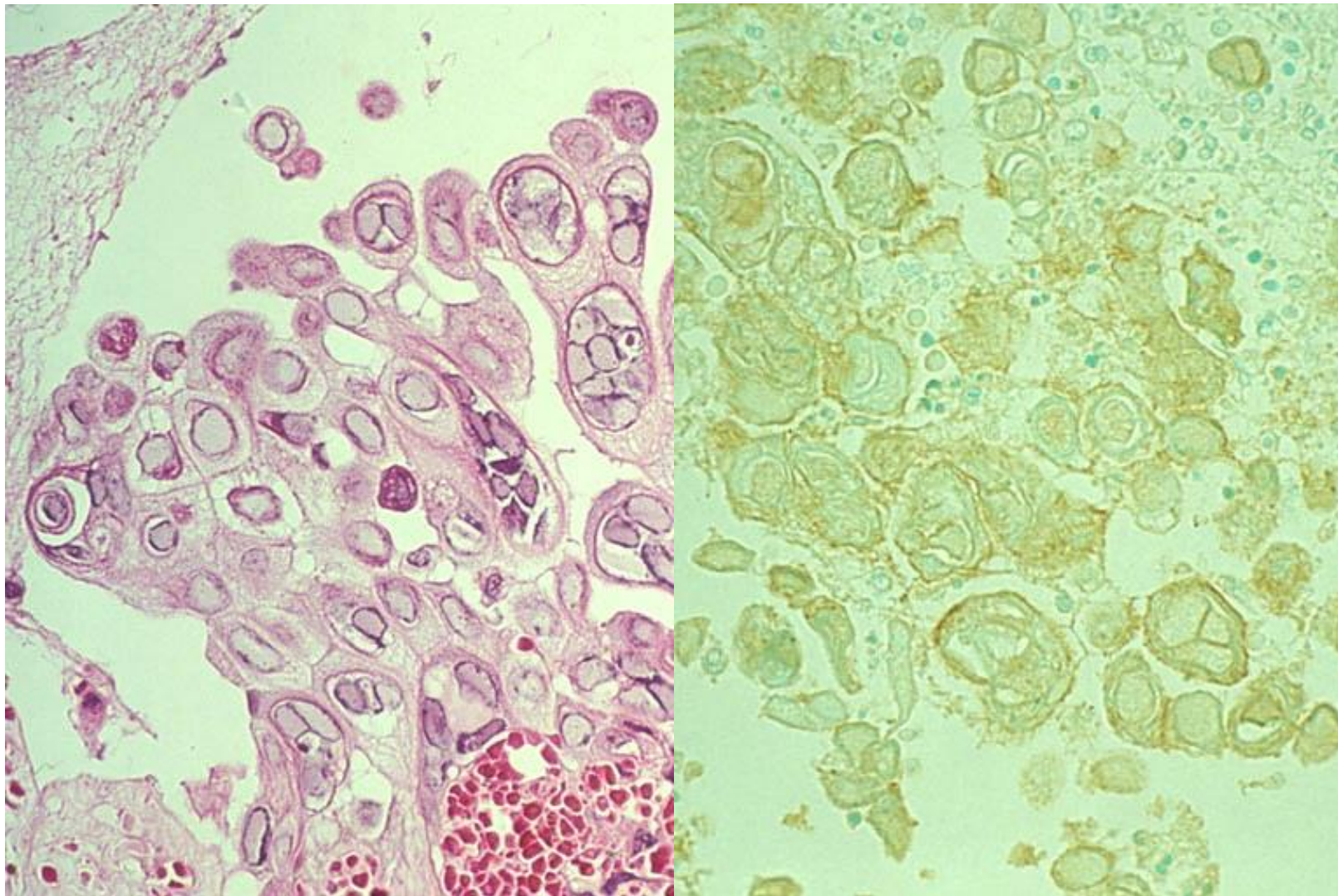
Splenic ascariasis (left, cut surface of the formalin-fixed spleen; center, H&E; right, reactivity with the serum of patient with hepatic ascariasis). Ectopic migration of *Ascaris lumbricoides* has provoked necrotizing granulomas beneath the splenic capsule. Dead parasite body fragments are seen in the necrotic area. The above-mentioned serum of patient with hepatic ascariasis reacts with the dead worm body, confirming the diagnosis of ectopic splenic ascariasis.



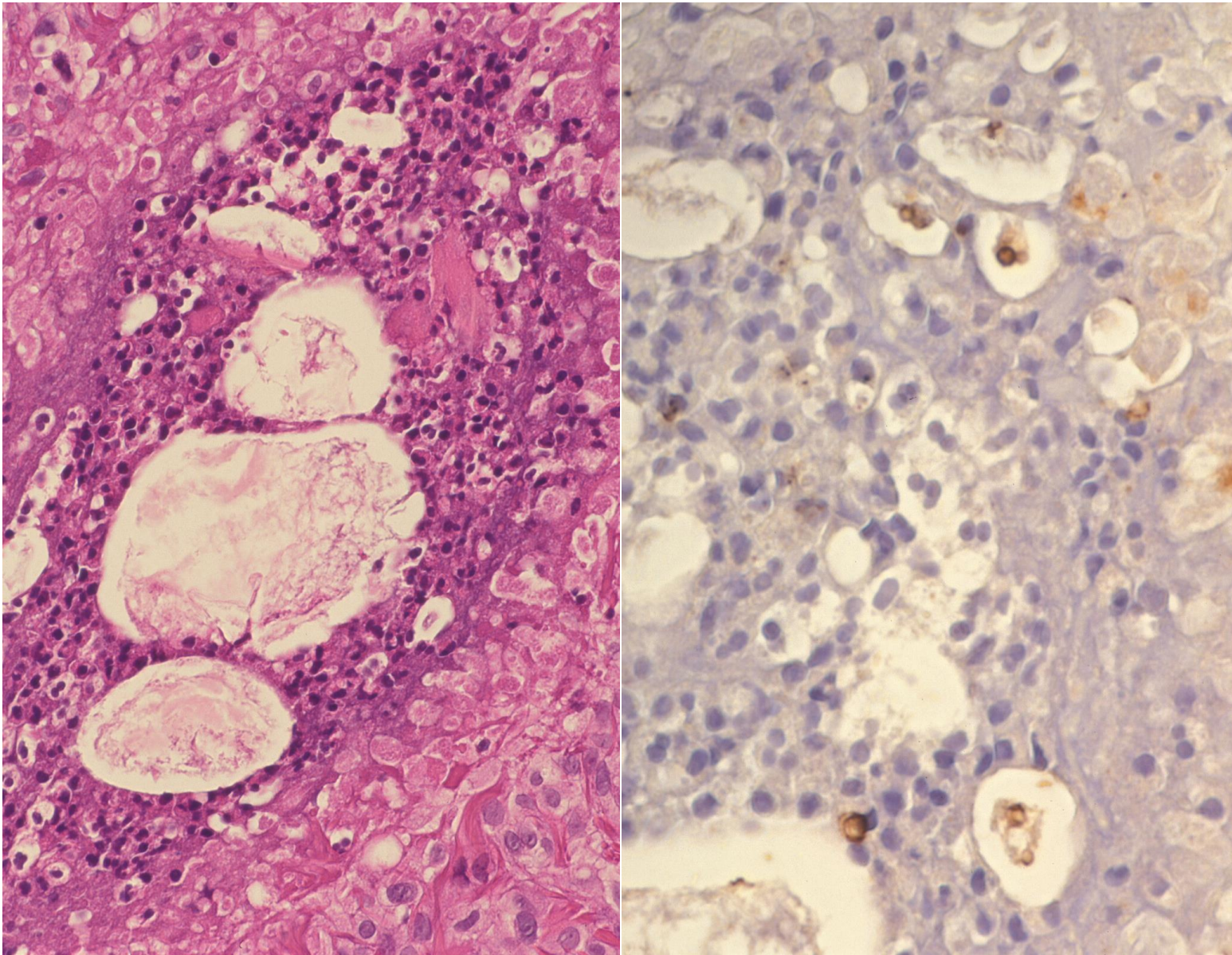
Gnathostomiasis. Left, H&E; right, reactivity with patient's own serum. A Japanese male manifested creeping disease on the abdomen. Targeted biopsy reveals cut surfaces of *Gnathostoma hispidum* in the dermis. The larval body is histologically featured by spiculated cuticles, well-developed muscle cells with clear cytoplasm, eosinophilic lateral chords, and the centrally located gut. The patient's serum was reactive with the gut cells.



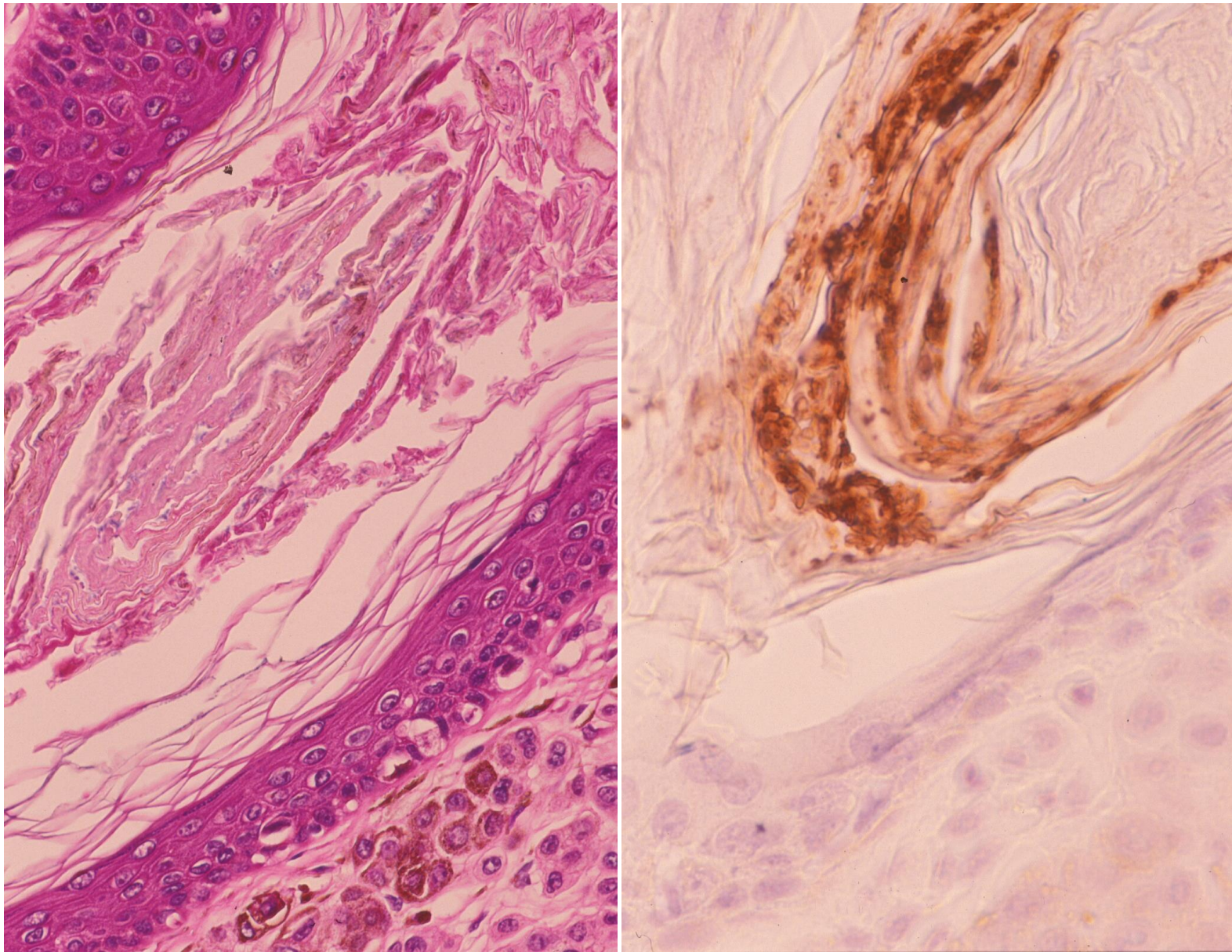
Schistosomiasis japonicum. Left, acid-fast staining; right, reactivity with patient's own serum. In biopsied colonic mucosa of an asymptomatic individual living in a historical endemic area, acid-fast ovum of *Schistosoma japonicum* is observed in the lamina propria mucosae. The patient's serum diluted at 1:200 reacts with the shell of the ovum.



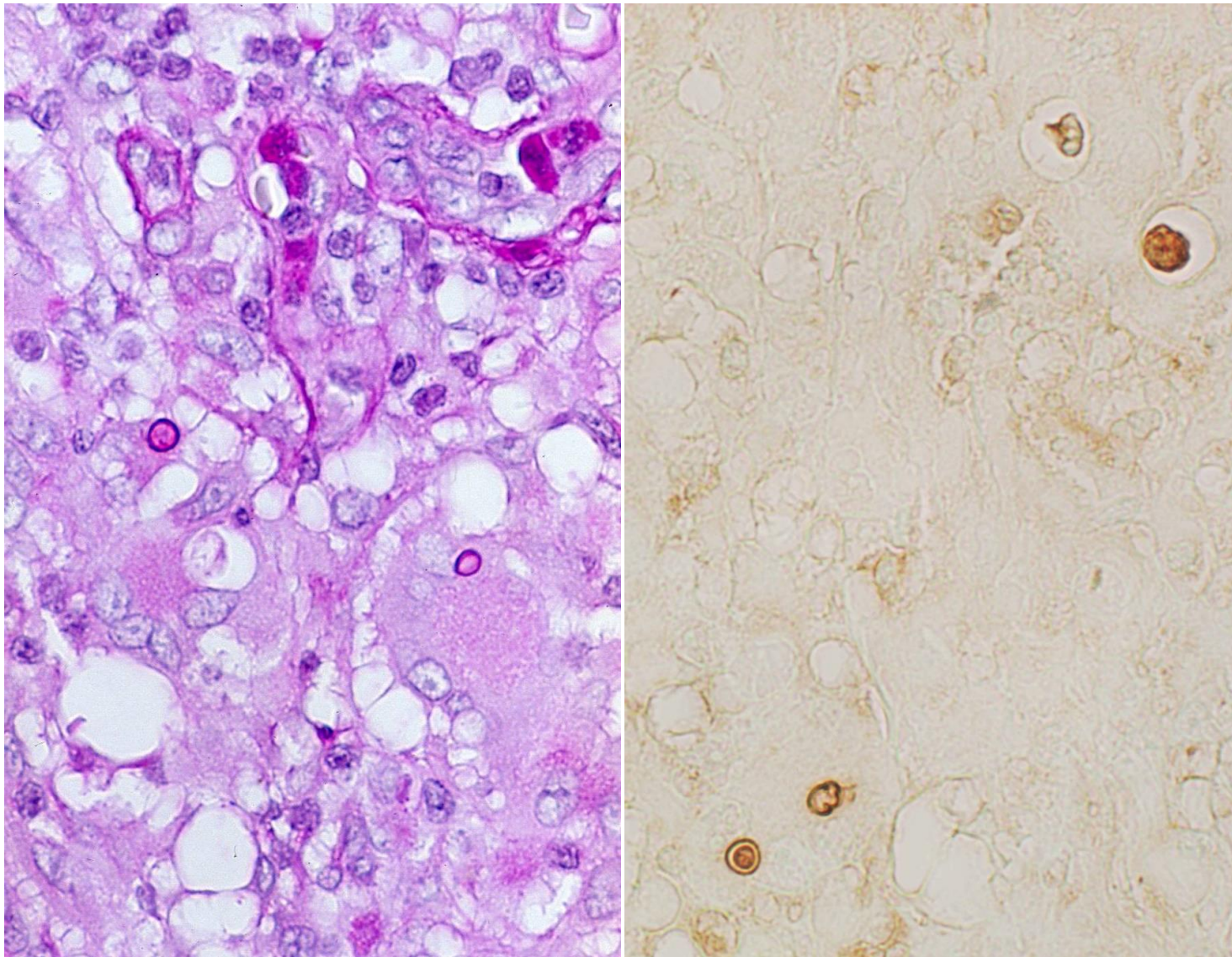
Hemorrhagic varicella complicated with pediatric acute lymphoblastic leukemia treated by bone marrow transplantation. Systemic dissemination of varicella-zoster virus accompanied multifocal hemorrhagic bullae on the skin and lethal varicella pneumonia. The bullous lesion show intranuclear viral inclusions (left: H&E), and a 1:500 diluted serum from an adult with herpes zoster decorates the plasma membrane of the viral infected keratinocytes, reacting with an immunodominant glycoprotein antigen GP-1 (right). **Ref.:** Suwabe H, et al. Relapsing hemorrhagic varicella. *Pathol Int* 1996; 46(8): 605-609. doi: 10.1111/j.1440-1827.1996.tb03661.x



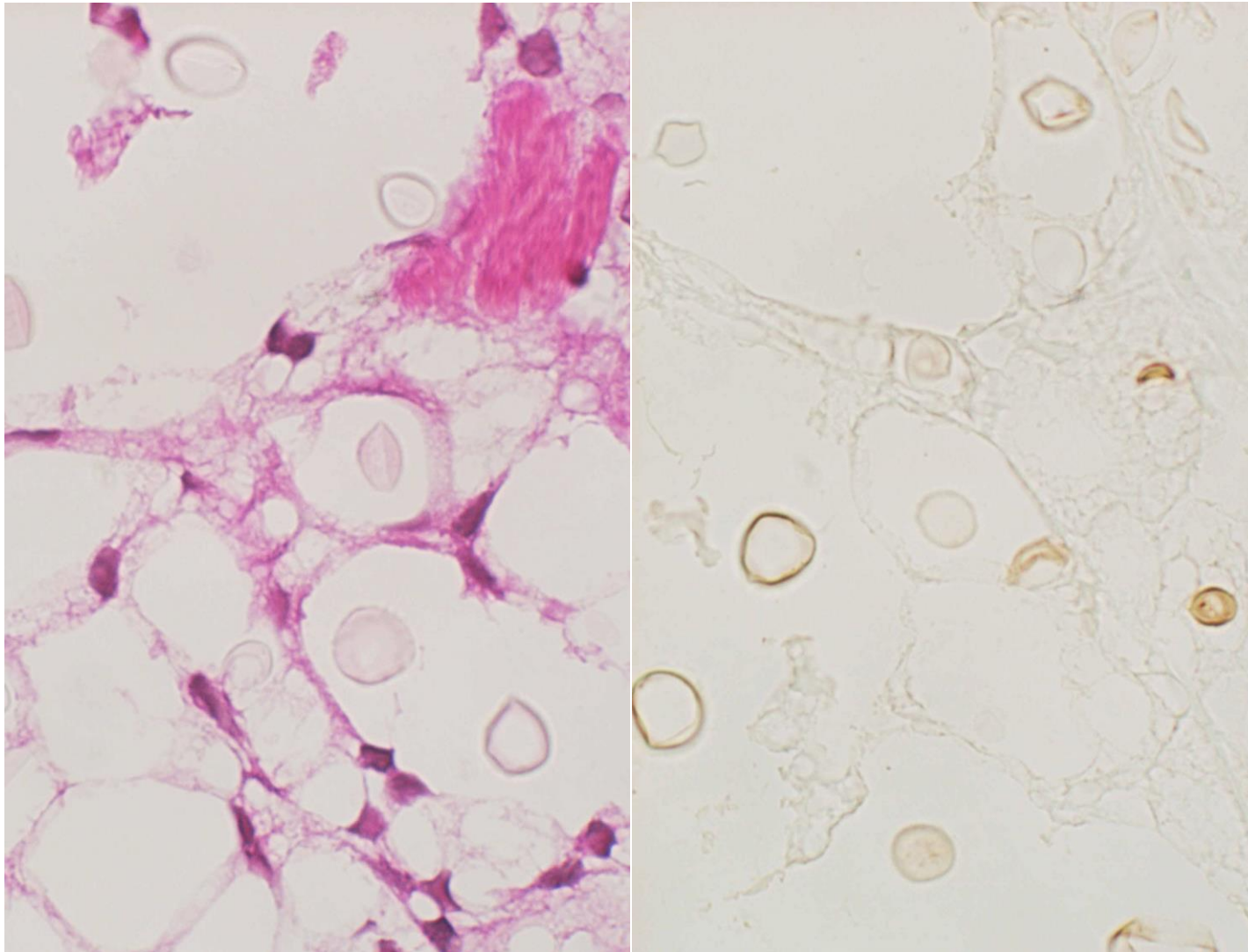
Malassezial folliculitis. Hair follicle-centered acute inflammatory change with mild granulomatous reaction is observed (left: H&E). Yeast-type fungi can be visualized with the indirect immunoperoxidase staining using 1:1,000 diluted patient's own serum (right).



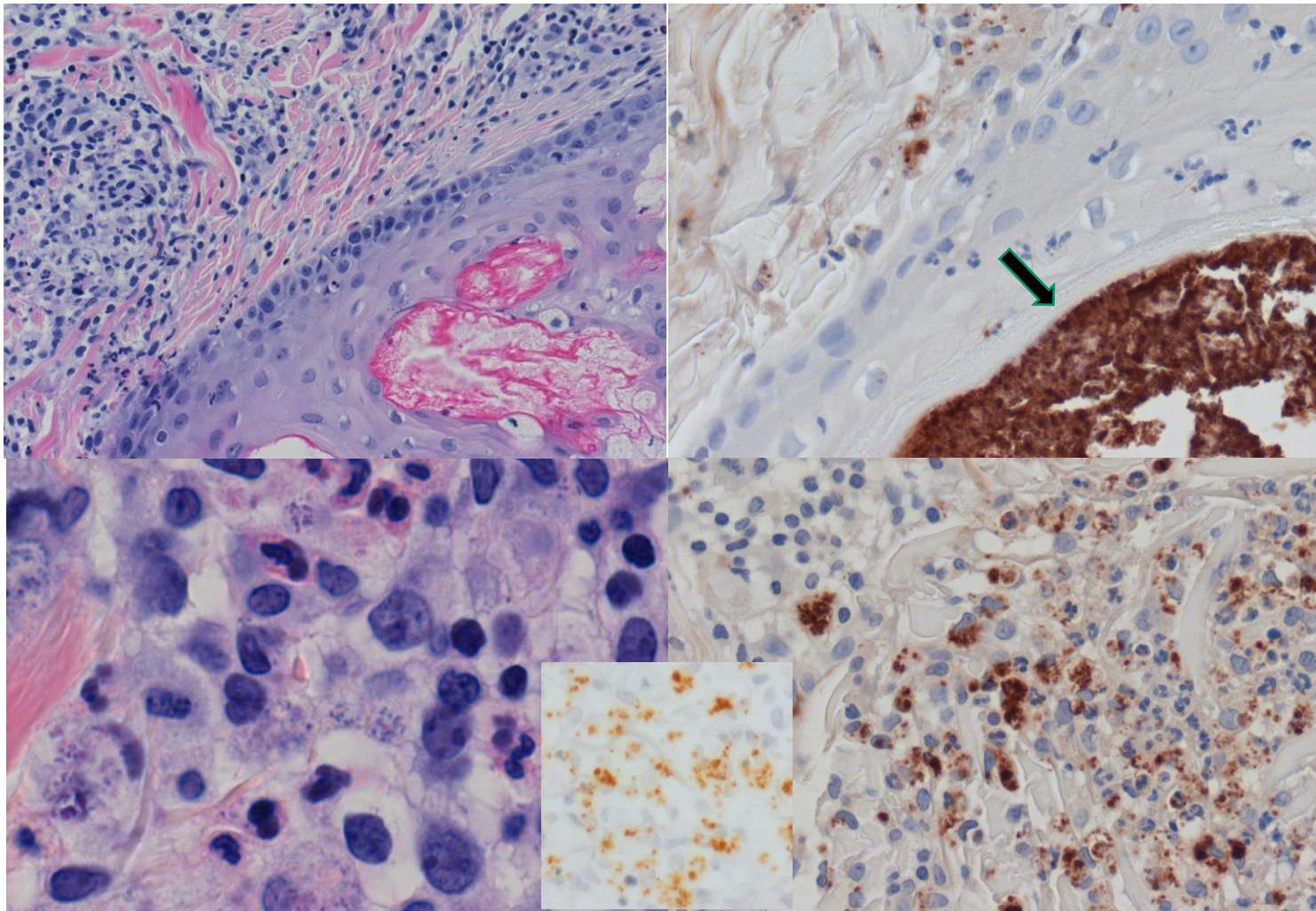
Commensal *Melassezia furfur* in the cornified layer on intradermal nevus biopsied from the facial skin (left: H&E). The diluted serum of the patient with Malassezian folliculitis decorate the resident fungi on the skin (right).



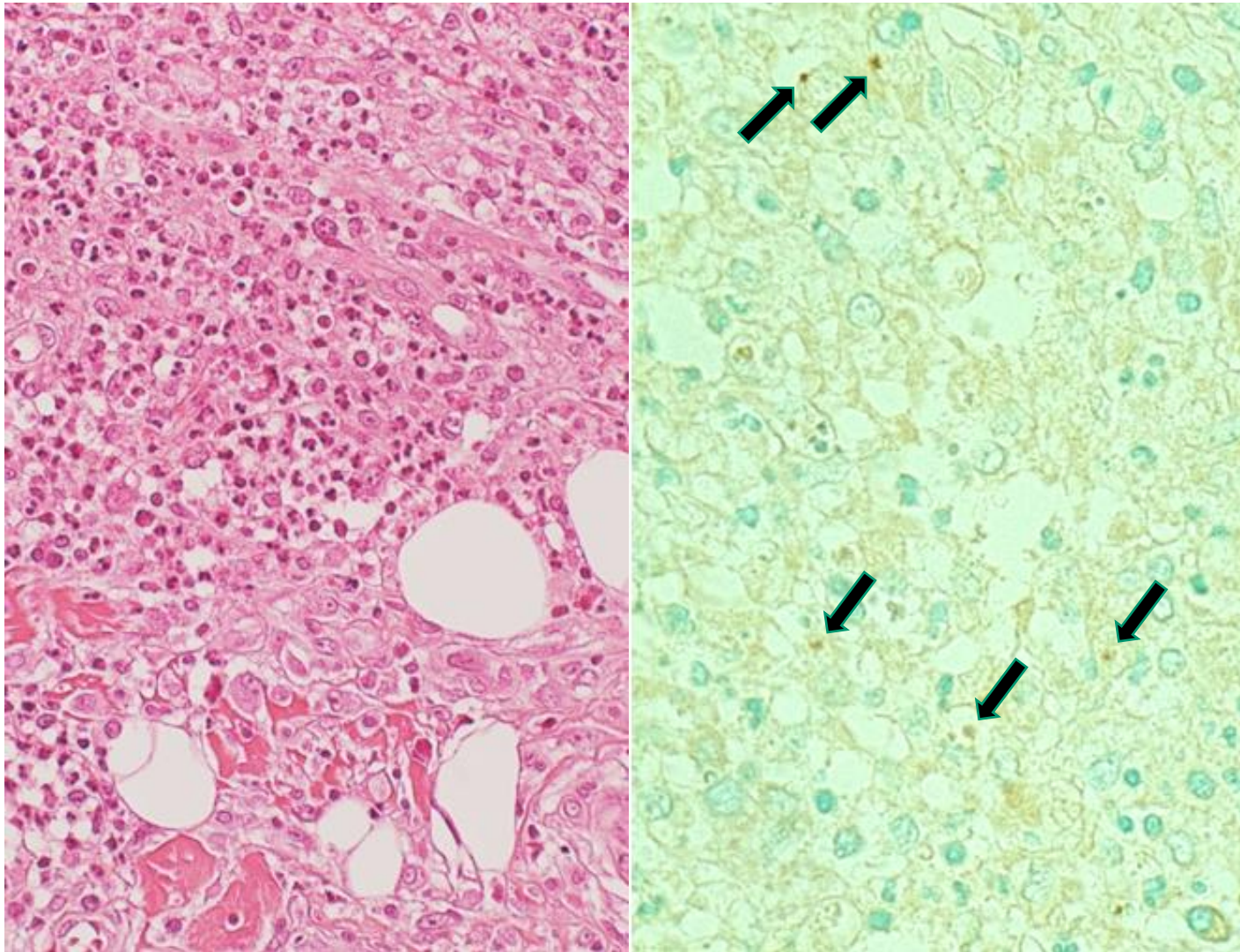
Sporotrichosis (left, periodic acid-Schiff reaction; right, reactivity with patient's own serum). Suppurative granulomas are formed in the dermis. Periodic acid-Schiff (PAS)-reactive small yeast-like fungal cells are phagocytized by macrophages or multinucleated giant cells, and are reactive with the patient's own serum.



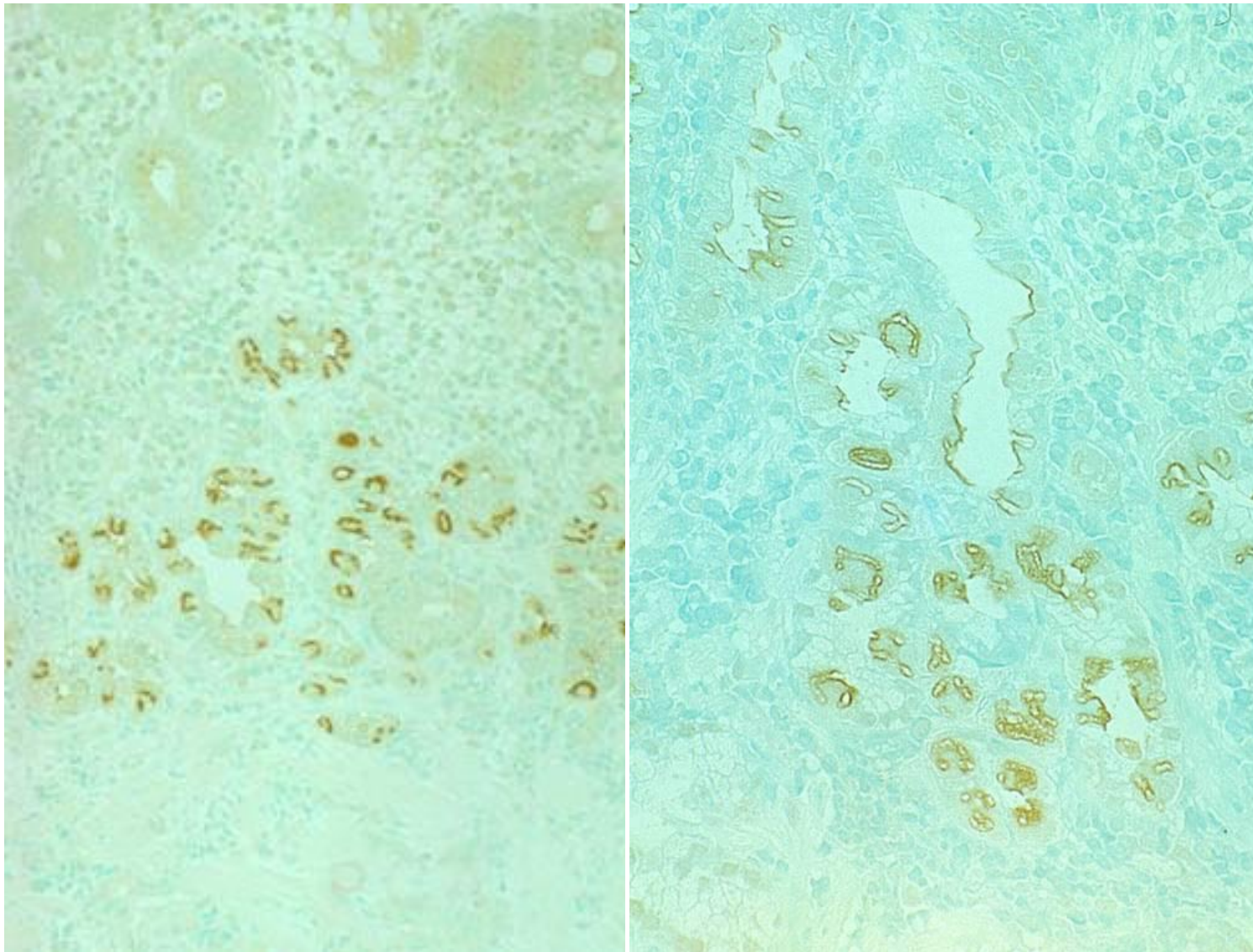
Cutaneous cryptococcosis (left, H&E; right, reactivity with patient's own serum). Opportunistic skin infection of *Cryptococcus neoformans* in a treated leukemia case of the young microscopically reveals transparent yeasts floating in mucoid material. Inflammatory reaction is poor. The patient's own serum diluted at 1:10 weakly reacts to the pathogen.



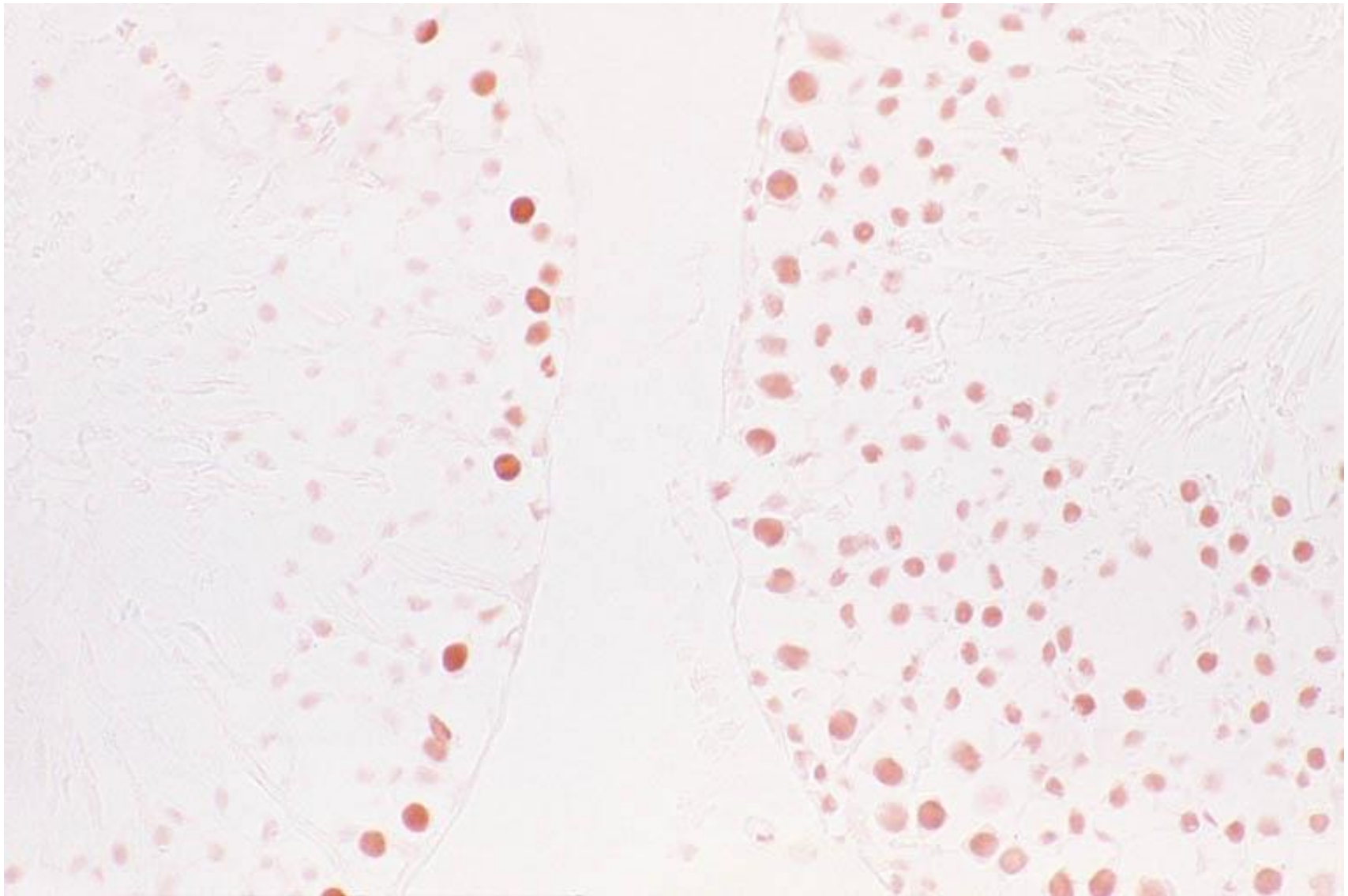
***Propionibacterium acnes* folliculitis.** Upper panels, dilated hair follicle with histiocytic reaction in the perifollicular dermis; lower panels, higher powered view of the infiltrating macrophages; left, H&E; right, reactivity with patient's own serum; inset, immunostaining for PAC3 antigen specific to *P. acnes*. Macrophages clustered around the dilated hair follicle contain numerous granular-looking microbes. The patient serum clearly demonstrates the pathogen not only in macrophages but also in commensal bacterial colonies in the hair follicle (arrow). Immunostaining for PAC3 antigen confirms the diagnosis of *P. acnes*-induced folliculitis



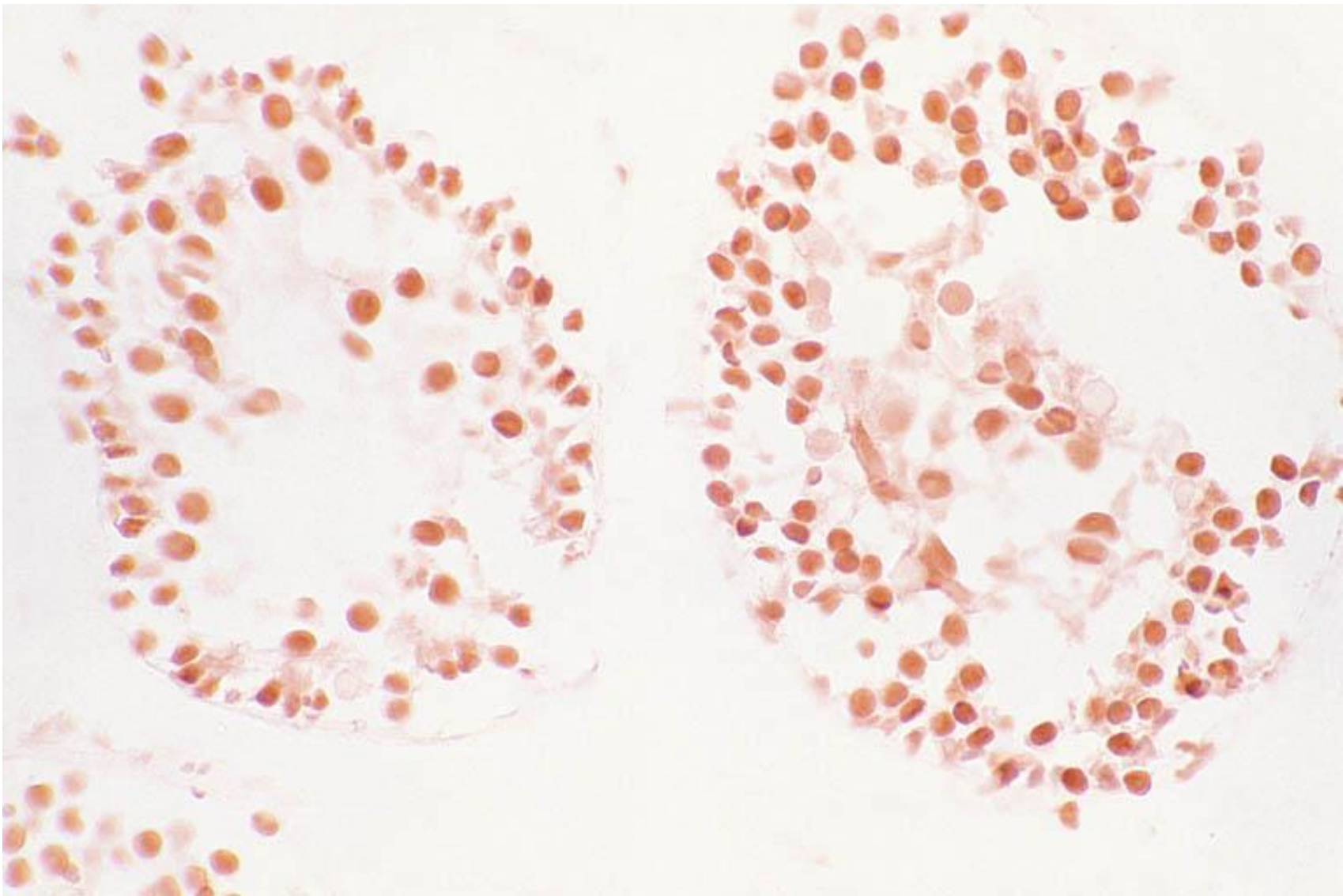
***Staphylococcus aureus*-induced pyoderma** (left, H&E; right, reactivity with patient's own serum). The dermis reveals diffuse infiltration of neutrophils and macrophages. The patient's own serum detects coccoid bacteria phagocytized by the inflammatory cells (arrows).



Parietal cell antibody from the serum of a patient with autoimmune (type A) gastritis was used for the indirect immunoperoxidase demonstration of normal parietal cells. C-shaped secretory canaliculi are clearly immunostained with the patient's serum. **Ref.:** Tsutsumi Y, Hara M. Application of parietal cell autoantibody to histopathological studies. *Acta Pathol Jpn* 1985; 35(4): 823-829. doi: 10.1111/j.1440-1827.1985.tb00624.x



PCNA (proliferating cell nuclear antigen). Application of the SLE patient's serum with anti-PCNA activity. In a PLP-fixed frozen section of the normal rat testis, the nuclei of spermatogonia and differentiated cells are labeled. It should be noted that the antiserum has no species specificity, and the immunostaining is applicable to the insect and vegetable tissues.



PCNA (proliferating cell nuclear antigen). Application of the SLE patient's serum with anti-PCNA activity. In a PLP-fixed frozen section of the atrophic rat testis after prolonged administration of estradiol, most nuclei are labeled. It should be noted that PCNA remain in the nuclei after the cessation of mitotic activity.

Summary

In the patient who has microscopically confirmed abscess or granuloma in the infected focus, IgG molecules with high antibody titers are expected in the serum, and the diluted serum can be applied as a primary antibody to detecting pathogens in formalin-fixed, paraffin-embedded sections. The application is particularly useful when the specific antibody is not available from commercial sources. The specificity of the patient's serum is unexpectedly high in cases of protozoan and helminthic infections.