SHORT REPORT

Marsh stars in Liverpool

A Agarwal, T R Helliwell

J Clin Pathol 2005;58:1104–1106. doi: 10.1136/jcp.2005.026187

Introduction/Methods: After the recognition of contaminating algae on histopathological sections stained by periodic acid Schiff (PAS) and Grocott methods, a detailed audit was undertaken to assess the extent of contamination and its possible source.

Results: The contaminating organism was a member of the staurastrum genus of Chlorophyta, star shaped organisms commonly found in fresh water marshes. The organisms were seen on sections stained by the diastase-PAS, PAS, or Grocott methods and on cytological preparations between July 2003 and May 2004. It is unlikely that contamination of water baths or concentrated staining solutions was to blame, and a more general contamination of the laboratory water supply is the most likely source.

Conclusions: Contaminating organisms may appear on histological and cytological material and their nature and source should be investigated. Although in this instance, confusion with pathologically important organisms was minimal, algae may occasionally cause significant disease.

Cellular pathology laboratories rely on a range of stains to colour the tissues and make diagnostic features apparent to the observer. Staining artefacts are well described^{1,2} and include uneven staining or clumped stain deposits, in addition to staining of inappropriate components of the tissues when methods are not correctly followed. Occasionally, microorganisms may be introduced on to tissue sections and this can lead to diagnostic difficulty. In our laboratory, a contaminant was noticed on sections stained with the periodic acid Schiff (PAS) reagent or Grocott silver method (fig 1) during 2003 and early 2004, first appearing in July 2003. Here, we describe the results of an audit that was performed to identify the contaminant and to determine its possible origin.

“Occasionally, microorganisms may be introduced on to tissue sections and this can lead to diagnostic difficulty”

METHODS

The laboratory database (Telepath) was searched for all cases on which PAS, diastase-PAS, or Grocott stains had been performed from June to September 2003. All the slides were reviewed for the contaminant. A 10% sample of cases from October 2003 to June 2004 was reviewed to assess the duration of the contaminating episode.

RESULTS

One hundred and eighty one slides were reviewed from 101 cases (June–September 2003) and the contaminant was seen on 44 slides (table 1).

The contaminating organisms stained magenta with the PAS and diastase-PAS methods and were positive with the Grocott stain (fig 1A–C). The organism was usually seen as a

pair of semi-cells in variable configuration. Individual cells had triradial symmetry with elongated diverging processes, which were minutely trifid at the apex. The processes were rough with minute granules arranged in transverse lines. Comparison with published images identified the contaminant as an algae of the staurastrum genus, most probably *Staurastrum paradoxum*.^{3,4}

Work in our laboratory is split into diagnostic teams, with each team having its own microtomes and water baths, and a common staining area. The contaminant was seen on sections of liver, skin, lung, lymph nodes, duodenum, and oesophagus that are handled by different teams. It was also identified on Papanicolaou and Geimsa stained cytology slides at the same time (fig 1D), although these have not been systematically reviewed. The contaminant first appeared in July 2003 and was present in both histology and cytology laboratories until May 2004.

The source of contamination seems unlikely to be the water baths used for floating out tissue sections, because these are emptied and cleaned regularly and no one water bath could account for the presence of the contaminant on a variety of tissue types. In addition, water bath contamination would not account for the organisms in cytological preparations. Repeated sampling of the concentrated staining reagents did not reveal these organisms. The most probable source is the main water supply common to both cytology and histopathology laboratories because the algae were seen at the same time and disappeared at approximately the same time from both the histology and cytology laboratories.

DISCUSSION

The contaminating organism seen in our study is a member of the staurastrum genus of algae, most probably *Staurastrum paradoxum*. These are Chlorophytes of the family Desmidiaceae, and are fresh water algae typically found in marshy water growing at 10–15°C. The star-like, triradial symmetry is typical of staurastrum.^{3,4} The more complex types of desmid algae are divided by an isthmus into two semi-cells and may form long chains (desmos is Greek for a bond or chain). Cell division is through the isthmus. Like other Chlorophytes they synthesise starch, and are also covered in a thin layer of mucilage; these features account for the staining characteristics noted in the laboratory.

“The possibility of contamination should always be kept in mind when reporting histology and cytology preparations”

Contamination of histological and cytological slides with exogenous material such as cloth fibres and starch from glove powder is relatively common, and contamination with microorganisms, particularly bacteria and fungi, is unusual. We are not aware of a previous report of algal contamination in a diagnostic laboratory.

Abbreviations: PAS, periodic acid Schiff

Figure 1 *Staurastrum paradoxum* present on the surface of sections stained by (A, B) diastase periodic acid Schiff, (C) Grocott, and (D) Giemsa methods. The organisms have triradial symmetry and semi-cells are seen in pairs. Scale bars are 100 µm.

The possibility of contamination should always be kept in mind when reporting histology and cytology preparations. It is important to identify the contaminant and locate the source as far as possible, and to distinguish it from pathogens. The source of contamination in this instance was not specifically identified, although we felt that sources within the laboratory environment itself had effectively been excluded.

Table 1 Summary of results			
	Total no. slides	Contaminant present	Proportion contaminated
PAS	95	25	26%
DPAS	67	15	22%
Grocott	19	4	21%
Total	181	44	24%

DPAS, diastase-periodic acid Schiff; PAS, periodic acid Schiff.

Take home messages

- We identified a contaminating organism in our histological and cytological material as a member of the staurastrum genus of Chlorophyta, star shaped organisms commonly found in fresh water marshes
- The organisms were seen on sections stained by the diastase-periodic acid Schiff (PAS), PAS, or Grocott methods and on cytological preparations between July 2003 and May 2004
- Our results indicate that it is unlikely that contamination of water baths or concentrated staining solutions was to blame, and a more general contamination of the laboratory water supply is the most likely source
- Although in this instance, confusion with pathologically important organisms was minimal, algae may occasionally cause significant disease

Algae are uncommon human pathogens, with cutaneous or gastrointestinal disease most commonly caused by immersion in, or consumption of, contaminated water. Protothecosis is caused by a ubiquitous achlorophyllous algae, and may lead to cutaneous and subcutaneous infection, synovitis or bursitis, or systemic infection.^{5 6} Paralytic and ciguatera fish poisoning result from eating fish that have accumulated ciguatoxin and related toxins from dinoflagellates.⁷ Symptoms include nausea, vomiting, and neurological symptoms such as paralysis and paraesthesiae.

Authors' affiliations

A Agarwal, T R Helliwell, Department of Pathology, Royal Liverpool University Hospitals NHS Trust, Duncan Building, Daulby Street, Liverpool L69 3GA, UK

Correspondence to: Dr T R Helliwell, Department of Pathology, Royal Liverpool University Hospitals NHS Trust, Duncan Building, Daulby

Street, Liverpool L69 3GA, UK; trh@liv.ac.uk

Accepted for publication 4 February 2005

REFERENCES

- 1 **Stevens A**. Pigments and minerals. In: Bancroft JD, Stevens A, eds. *Theory and practice of histological techniques*. Edinburgh: Churchill Livingstone, 1990:245–6.
- 2 **Culling CFA**, Allison RT, Barr WT. *Cellular pathology techniques*, 4th ed. London: Butterworths, 1985.
- 3 Desmids Dot Com (<http://www.sitewiz.co.uk/sites/desmids.com>) last accessed 27 November 2004.
- 4 **Ralfs J**. The British Desmidiaceae—Staurastrum. 1848 (<http://www.biologie.uni-hamburg.de/b-online/ralfs/119.htm>) last accessed 27 November 2004.
- 5 **Thiele D**, Bergmann A. Protothecosis in human medicine. *Int J Hyg Environ Health* 2002;**204**:297–302.
- 6 **Torres HA**, Bodey GP, Tarrand JJ, et al. Protothecosis in patients with cancer: case series and literature review. *Clin Microbiol Infect* 2003;**9**:786–92.
- 7 **Sakamoto Y**, Lockey RF, Krzanowski JJ. Shellfish and fish poisoning related to the toxic dinoflagellates. *South Med J* 1987;**80**:866–72.