to survive longer than other enteroviruses and are a good indication of the quality of the water. Although viruses were detected in samples taken from various points during reclamation before the final chlorination stage, it would appear that the final water from the reclamation plant is completely free of viral contamination and is as clear as domestic tap-water. The fact that there was a heavier deposit of discoloured matter on the membrane filter after tap-water had been concentrated would be because of its storage and passage through many pipes.

By virological standards the final water from the Cape Flats reclamation plant is perfectly potable.

The results presented here confirm those reported by the National Institute for Water Research of the South African Council for Scientific and Industrial Research1 on its studies of a similar pilot plant in Pretoria (Stander Plant) and those reported by Isaacsen et al.3 for the Windhoek reclamation plant.

REFERENCES


Blastomyces dermatitidis infections in the RSA


Summary

Twenty cases of blastomycosis have been confirmed in the RSA, 9 of which are presented for the first time. Patients came from all four provinces and the mean age was 40 years. Six cases were diagnosed between 1985 and 1987. Differences between strains of Blastomyces dermatitidis isolated in the RSA and in North America include morphological and cultural characteristics, mycelial-yeast conversion, antigenic structure, and compatibility in cross-mating experiments. The diagnosis of this disease can be made by direct examination of unstained specimens, by histological examination or by culture of the organism. Culture should be attempted in all cases for confirmation of microscopic findings. Six cases were diagnosed between 1985 and 1987. Differences between strains of Blastomyces dermatitidis isolated in the RSA and in North America include morphological and cultural characteristics, mycelial-yeast conversion, antigenic structure, and compatibility in cross-mating experiments. The diagnosis of this disease can be made by direct examination of unstained specimens, by histological examination or by culture of the organism. Culture should be attempted in all cases for confirmation of microscopic findings.

Blasramyces dennariridis.

The studies were financed by the Water Research Commission and their financial assistance is gratefully acknowledged.

The portal of entry of the infectious conidia is the lung, from where dissemination occurs, mainly to skin and bone. The kidney and prostate are other organs commonly involved. In the lung, the disease process may be acute or chronic, the latter presenting in a similar fashion to tuberculosis or carcinoma. Involvement of almost every tissue has been documented.

A number of reports of blastomycosis have appeared in the SA MF recently.3-5 We contacted microbiologists and clinicians in major centres in South Africa (Johannesburg, Pretoria, Bloemfontein, Cape Town and Durban) and obtained details of 9 unpublished cases, which have occurred over the last 10 years, bringing to 20 the total number of blastomycosis cases reported in this country since 1959.4

A survey of published reports showed that 57 cases have been described in African countries other than South Africa since the first report in 1952.3 Of these, 28 occurred in Zimbabwe,5-10 5 in Zaire,11-14 4 in Morocco,15-17 3 in Tunisia,18 3 in Zambia,19 2 each in Mozambique,20-22 2 each in Egypt,23 1 each in Algeria,24 1 each in Algeria,25 1 each in Algeria,26 1 each in Zambia,19 1 each in Mozambique,20-22 1 each in Egypt,23 1 each in Algeria,24 1 each in Algeria,25 1 each in Algeria,26 1 each in Zambia,19 1 each in Mozambique,20-22 1 each in Egypt,23 1 each in Algeria,24 1 each in Algeria,25 1 each in Algeria,26 1 each in Zambia,19 1 each in Mozambique,20-22 1 each in Egypt,23 1 each in Algeria,24 1 each in Algeria,25 1 each in Algeria,26 1 each in Zambia,19 1 each in Mozambique,20-22 1 each in Egypt,23 1 each in Algeria,24 1 each in Algeria,25 1 each in Algeria,26 1 each in Zambia,19 1 each in Mozambique,20-22 1 each in Egypt,23 1 each in Algeria,24 1 each in Algeria,25 1 each in Algeria,26 1 each in Zambia,19 1 each in Mozambique,20-22 1 each in Egypt,23 1 each in Algeria,24 1 each in Algeria,25 1 each in Algeria,26 1 each in Zambia,19 1 each in Mozambique,20-22 1 each in Egypt,23 1 each in Algeria,24 1 each in Algeria,25 1 each in Algeria,26 1 each in Zambia,19 1 each in Mozambique,20-22 1 each in Egypt,23 1 each in Algeria,24 1 each in Algeria,25 1 each in Algeria,26 1 each in Zambia,19

In addition, we know of 1 Mozambican, 1 South West African/Namibian and 2 Zimbabwean cases that have not been published. Thus, to date, the total number of cases reported from Africa is 81.

Examples of South African cases which illustrate the clinical features and treatment of the infection can be found in recent publications,1,2 but little has been written about the causative organism. Therefore what is known of the epidemiology of the disease in the RSA, the comparative mycology of the fungal strains responsible for the disease in Africa and North America, and information to help both those submitting and those processing specimens to make the diagnosis of blastomycosis is presented.
TABLE I. SOUTH AFRICAN CASES OF BLASTOMYCOSIS

<table>
<thead>
<tr>
<th>Province</th>
<th>Year of diagnosis</th>
<th>Age (yrs)</th>
<th>Sex</th>
<th>Race</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cape Province</td>
<td>1985</td>
<td>40</td>
<td>M</td>
<td>B</td>
<td>Unpublished</td>
</tr>
<tr>
<td>Orange Free State</td>
<td>1961</td>
<td>29</td>
<td>M</td>
<td>B</td>
<td>Emmons et al. 28</td>
</tr>
<tr>
<td></td>
<td>1983</td>
<td>34</td>
<td>M</td>
<td>B</td>
<td>Unpublished</td>
</tr>
<tr>
<td></td>
<td>1975</td>
<td>A</td>
<td>M</td>
<td>B</td>
<td>Unpublished</td>
</tr>
<tr>
<td></td>
<td>1985</td>
<td>29</td>
<td>M</td>
<td>B</td>
<td>Berkowitz and Diamond 2</td>
</tr>
<tr>
<td>Natal</td>
<td>1968</td>
<td>36</td>
<td>M</td>
<td>W</td>
<td>Martin and Berson 4</td>
</tr>
<tr>
<td></td>
<td>1970</td>
<td>39</td>
<td>M</td>
<td>B</td>
<td>Osmond et al. 28</td>
</tr>
<tr>
<td></td>
<td>1973</td>
<td>17</td>
<td>F</td>
<td>?</td>
<td>Unpublished</td>
</tr>
<tr>
<td></td>
<td>1978</td>
<td>65</td>
<td>M</td>
<td>?</td>
<td>Unpublished</td>
</tr>
<tr>
<td></td>
<td>1986</td>
<td>44</td>
<td>M</td>
<td>B</td>
<td>Allison and McCallum 36</td>
</tr>
<tr>
<td>Transvaal</td>
<td>1959</td>
<td>50</td>
<td>F</td>
<td>B</td>
<td>Martin and Berson 4</td>
</tr>
<tr>
<td></td>
<td>1967</td>
<td>27</td>
<td>M</td>
<td>B</td>
<td>Martin and Berson 4</td>
</tr>
<tr>
<td></td>
<td>1975</td>
<td>46</td>
<td>M</td>
<td>B</td>
<td>Fragoyannis et al. 29</td>
</tr>
<tr>
<td></td>
<td>1975</td>
<td>63</td>
<td>M</td>
<td>W</td>
<td>Simon et al. 40</td>
</tr>
<tr>
<td></td>
<td>1975</td>
<td>?</td>
<td>M</td>
<td>B</td>
<td>Unpublished</td>
</tr>
<tr>
<td></td>
<td>1984</td>
<td>37</td>
<td>M</td>
<td>B</td>
<td>Unpublished</td>
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<tr>
<td></td>
<td>1985</td>
<td>45</td>
<td>M</td>
<td>B</td>
<td>De Villiers et al. 5</td>
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<tr>
<td></td>
<td>1985</td>
<td>47</td>
<td>M</td>
<td>W</td>
<td>Hurwitz et al.</td>
</tr>
<tr>
<td></td>
<td>1987</td>
<td>26</td>
<td>M</td>
<td>B</td>
<td>Unpublished</td>
</tr>
</tbody>
</table>

We are aware of 1 more unpublished case involving a black male which was diagnosed during 1975 - 1985 but no other details are available.

A = adult; F = female; M = male; B = black; W = white.

**Epidemiology**

Table I shows some epidemiological data concerning South African cases of blastomycosis. Cases have been diagnosed in all provinces, with the Transvaal the best represented; this province is also the most populous. However, in other regions of the world the incidence is more closely related to exposure to environmental conditions favouring sporulation of the fungus than to the population size. It may be that from time to time suitable conditions exist in the Transvaal more often than in the other provinces. These conditions are usually found in circumscribed sites; an example is the recently described outbreak associated with a beaver lodge in Wisconsin. 30 A localised outbreak such as this has not been described in South Africa; however, 2 patients, widely separated in time, are known to have come from Welkom in the Orange Free State (OFS). Similarly, 2 cases of blastomycosis occurred in patients living in the Mafikeng area of the western Transvaal. There seems to be an inverse relationship between latitude and incidence; only 1 case has been diagnosed in the Cape Province, 4 in Natal, 5 in the OFS, 9 in the Transvaal, and more than 25 cases have been described from Zimbabwe. 6-10 The organism has not been recovered from the environment in southern Africa, and even in North America this has been accomplished only a few times. 30

Fig. 1 shows the distribution of the cases over time. It is possible that the conditions of temperature and humidity necessary for sporulation of the organism were more often present during some periods than during others, leading to peaks in incidence such as occurred during the years 1985 - 1987.

The age of the patients ranges from 17 years to 65 years, with a mean of 40 years. Only 2 patients have been women, which may be a reflection of a greater exposure to the fungus in outdoor occupations of men. Since outdoor exposure is considered to be a risk factor, it could be expected that blacks would have a much greater incidence than whites, especially in agricultural areas. However, the ratio of black to white patients is only 4:1 in this series, i.e. lower than the ratio in the population as a whole. This might be explained either by the fact that black people do not present to hospital as often as whites or by misdiagnosis.

**Comparative mycology**

A number of authors have addressed the problem of whether the North American and African isolates of *B. dermatitidis* represent different species or merely different serotypes. Morphological differences have been described: cultures of North American isolates tended to be velvety white becoming tan in colour, with yeast cells 8 - 12 µm in diameter. In contrast, African isolates were moist and glabrous with a furrowed mycelium, and yeast cells were 9 - 18 µm in diameter. 31 A tendency of the yeast forms of some African isolates to filamentation or hyphal element production, resulting in bizarre swollen cells, has been noted 31 and was also seen by one of the authors (C. N. Y. — unpublished observation).
Vermeil et al.32 pointed out that the ability of the mycelial phase to convert to the yeast phase was abolished or lessened in the African strains, in contrast with the American ones, which routinely achieve this conversion. Kaufman et al.31 succeeded in getting 7 of 11 African strains to convert fully.

Serological differences have been noted consistently; studies in which immunoprecipitation31 and immunofluorescence33 were used, showed that American isolates were antigenically more complex. Kaufman et al.31 found antigens which they designated A and K. The K antigen was common to all strains, regardless of geographic origin, whereas the A antigen was found in American, but not in African strains, apart from a solitary Algerian isolate. Single isolates from India and Israel also had the A antigen.

Cross-mating experiments, in which African and North American strains were paired, showed that true sexual spore formation did not occur,34 implying that the paired strains were not identical species.

These studies have been done on relatively small numbers of strains and more isolates need investigation to confirm these differences.

Diagnosis

The diagnosis of blastomycosis should be considered in patients who present with chronic subcutaneous abscesses or verrucous crusting skin lesions; who have bone abscesses tracking to the skin surface, particularly when the spine or ribs are involved; or who have chronic pulmonary disease not responding to antituberculous therapy.

The preferred specimens are pus, skin biopsies or scrapings, sputum and urine; fluid or biopsy material from any suspected site would, however, be appropriate. Biopsy material should be submitted both in formalin for histological examination and in normal saline for culture.

Since B. dermatitidis is a dimorphic fungus, the yeast forms are found in tissue. They are 7 - 20 μm in diameter, thick-walled, with characteristic single broad-based buds. Micro-abscesses or non-caseating granulomas are usually found. The organisms may be found free in the abscesses or inside giant cells. Haematoxylin and eosin staining will be adequate in most cases to make a diagnosis; however, special stains such as methenamine silver and periodic acid-Schiff (PAS) should be used for confirmation (Figs 2 and 3). Even with the methenamine silver stain it may be difficult to distinguish between small forms of B. dermatitidis and Histoplasma capsulatum. The PAS stain shows the multiple nuclei of B. dermatitidis, thus differentiating it from all other similar organisms which are uninucleate.

Potassium hydroxide (KOH) preparations may reveal the presence of the yeasts in pus and body fluids (Fig. 4). Papanicolaou staining of alcohol-fixed smears may demonstrate the organism.35 These rapid methods enable therapy to be started immediately, but irrespective of the results, culture should still be attempted as this is the only absolute diagnostic proof.36

The organism is readily cultured from fluids or biopsy material on standard mycological media, such as Sabouraud’s agar. Plates must be incubated at room temperature and at 37°C to demonstrate both mycelial and yeast phases respectively; the yeast phase requires enriched media such as blood agar to grow. Although growth may be detected within 10 days, plates should be kept for 8 weeks before being regarded as negative. In culture, the yeast forms are identical to those seen in specimens. The mycelium is white and fluffy, although it may become tan as the colony matures. Microscopically, thin septate branching hyphae are seen. Ovoid conidia (2 - 10 μm in size) are either sessile or borne on short lateral conidiophores.
Identification of the yeast phase can be confirmed by immunological means, e.g. immunofluorescence. Immunofluorescence can also be used to show the organism in tissue sections.64

Although not available in the RSA, serology has a role in diagnosis. A number of techniques, such as complement fixation and immunodiffusion, have been used for many years in the USA but these, although specific (100% for both), are not very sensitive (40% and 65% respectively, in one study).37 Recently, an enzyme-linked immunosorbent assay (ELISA), using purified yeast A antigen for the solid phase, has been described.37 This test, although marginally less specific (98%), had a sensitivity of 80% for human blastomycosis. The ELISA might be more suitable for serosurveys than the complement fixation or immunodiffusion tests. In the African context, we doubt whether this ELISA would be of value because of the absence of the appropriate antigen (see above) in African strains. A more useful ELISA for this region would require antigenic analysis of local strains.

In vitro lymphocyte transformation using an alkali- and water-soluble extract of the yeast phase has been used for the diagnosis of blastomycosis in an outbreak in the USA.30 The sensitivity of this test was 81% (specificity was not reported) and it may (with the ELISA) provide the only evidence of subclinical infection.

Skin testing with blastomycin, an extract of the mycelial phase of the fungus, is neither specific nor sensitive.

Conclusion

Although it is a rare disease in the RSA, blastomycosis should be entertained as a possible diagnosis when the clinician is faced with a patient suffering from chronic skin, lung or bone disease. The infection is relatively easily diagnosed if the correct specimens are submitted and laboratory personnel are aware of the condition.

Fortunately, blastomycosis is eminently treatable.

REFERENCES
