Seasonal variations in the prevalence of Cryptococcus neoformans var. grubii and Cryptococcus gattii in decayed wood inside trunk hollows of diverse tree species in north-western India: a retrospective study

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This study presents a 7-year retrospective analysis of seasonal variations in the prevalence of Cryptococcus neoformans var. grubii and Cryptococcus gattii in decayed wood inside trunk hollows of 518 trees belonging to 20 species in north-western India during 2000–2007. Of the 1,439 wood samples investigated, 406 (28.2%) were found to be positive for the Cryptococcus neoformans species complex which included 247 samples from which C. neoformans var. grubii was recovered and 171 which yielded C. gattii. While both of the pathogens were isolated throughout all the seasons, the overall prevalence of C. neoformans var. grubii was significantly higher (17.2%) than that of C. gattii serotype B (11.9%, P < 0.0001), indicating that decayed wood was as good, if not better, a natural habitat of C. neoformans var. grubii as that of C. gattii. The highest recovery of both yeasts was in the autumn, followed by that in the summer. For C. gattii, the lowest prevalence occurred during the winter and for C. neoformans var. grubii during the rainy season. The low prevalence of C. gattii during winter is similar to that reported from Bogota, Colombia, where C. gattii had a low population density in bark samples but it was not found in decayed wood of trunk hollows investigated during the period of January and February. The prevalence of C. neoformans var. grubii was significantly lower in the rainy season than in the other portions of the year. This finding is similar to the reported low isolation frequency (4%) of C. neoformans var. grubii from chicken feces in the rainy season in northern Thailand. Further investigations are warranted to determine the clinical significance of seasonal variations in the prevalence of C. neoformans var. grubii and C. gattii in decayed trunk wood of various trees in climatically divergent regions of India.

Key words Cryptococcus neoformans var. grubii, Cryptococcus gattii, seasonal prevalence, decayed wood, India

Introduction

In earlier publications [1–3], we reported that decayed wood inside trunk hollows of a wide spectrum of tree species and the soil in the vicinity of colonized trees in north-western India could serve as an important natural
reservoir for Cryptococcus neoformans var. grubii and Cryptococcus gattii, the principle etiologic agents of cryptococcosis. In addition, our preliminary investigations of a collection of C. neoformans var. grubii isolates from decayed trunk wood of trees in this region revealed evidence for long-distance dispersal and recombination [4]. The climate in north-western India varies widely from season to season including fluctuating temperature, relative humidity and rainfall. However, the effects of these climatic factors across the seasons on the prevalence of C. neoformans and C. gattii in decayed wood inside trunk hollows of various trees remain largely unknown. Here, we present a summary and retrospective analysis of our data on seasonal variations of the prevalence of both the pathogens in decayed wood sampled during a 7-year period, from 2000 to 2007.

Materials and methods

The data analyzed included the results from studies involving the isolation of C. neoformans and C. gattii from a total of 1,439 decayed wood samples collected from trunk hollows of 518 trees, representing 20 species. The investigated trees were distributed widely in the north-western states of Panjab, Haryana, U.P, and the Union Territories of Delhi and Chandigarh. Detailed information pertaining to the sites of collection and methods of sampling, strain isolation and identification of both C. neoformans and C. gattii can be found in our earlier publications [1–5]. In brief, sampling of decayed wood was done with the conventional sedimentation and an in-house swabbing technique, using simplified niger seed agar (NSA) as previously described by Randhawa et al. [5]. The colonies were purified by dilution plating and identified by their phenotypic features and verification of salient physiological characteristics, employing the VITEK 2 System (bioMérieux, Marcy l’Etoile, France). The isolates were then serotyped, using the Cryptococcus slide agglutination test (Iatron, Tokyo, Japan). Based on the parameters of temperature, relative humidity, rainfall and various phases in the life cycle of plants, the following five seasons have been recognized for the areas covered in our investigation.

Summer

From 21 April to 21 June the summer season is noted for very high temperatures, low relative humidity and low rainfall combined with desiccating high winds. Maximum temperatures are usually between 38°C and 42.5°C and may rise to 45°C and beyond on certain days. The minimum temperature varies between 21°C and 29°C. Ground vegetation is sparse.

Rainy

From 22 June to 22 September the rainy season is characterized by high temperatures and high relative humidity but with low diurnal ranges. The maximum temperature is usually between 34 and 40°C whereas the minimum temperature ranges from 25–29°C. About 80% of the total annual rainfall (~666.4 mm) occurs during this period. Ground vegetation is lush with the highest coverage during this period of the year.

Winter

The winter season lasts from 22 November to 19 February. Temperature and rainfall are both low. Relative humidity is moderate with a high diurnal range. The maximum temperature varies between 21°C and 24°C. Typical minimum temperatures range from 5–10°C but can reach freezing point on certain days.

Spring

From 20 February to 20 April the spring period is characterized by moderate temperature and moderate relative humidity. The maximum temperature is usually between 24 and 36°C whereas the minimum temperature ranges from 10–21°C. Rainfall is usually low.

The differences in prevalence between C. neoformans var. grubii and C. gattii across the seasons were analyzed, using the Chi-square contingency table test, with P values <0.05 considered as statistically significant.

Results and discussion

The summary of the results of the investigations of the seasonal dynamics of prevalence of the two Cryptococcus spp. are presented in Table 1 and depicted in Fig. 1. The variations in mean temperature, relative humidity and rainfall across the seasons in north-western India are shown in Fig. 2. Of the 1,439 wood samples investigated, 406 (28.2%) yielded members of the Cryptococcus neoformans species complex, which included 247 positive for C. neoformans var. grubii and 171 for C. gattii. Although both of the pathogens were isolated through all the seasons, the overall prevalence of C. neoformans var. grubii was significantly higher (17.2%) than that of C. gattii serotype.
Table 1  Seasonal variations in prevalence of Cryptococcus neoformans var. grubii and Cryptococcus gattii in decayed wood inside trunk hollows of miscellaneous trees in north-western India.

<table>
<thead>
<tr>
<th>Seasons</th>
<th>No. wood samples tested</th>
<th>No. positive for C. neoformans spp. complex (%)</th>
<th>No. positive for C. neoformans var. grubii (%)</th>
<th>No. positive for C. gattii (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Winter</td>
<td>481</td>
<td>128 (26.6)</td>
<td>84 (17.5)</td>
<td>44 (9.1)</td>
</tr>
<tr>
<td>Spring</td>
<td>275</td>
<td>65 (23.6)</td>
<td>39 (14.2)</td>
<td>26 (9.5)</td>
</tr>
<tr>
<td>Summer</td>
<td>176</td>
<td>64 (36.4)</td>
<td>42 (23.9)</td>
<td>28 (15.9)</td>
</tr>
<tr>
<td>Rainy</td>
<td>346</td>
<td>78 (22.5)</td>
<td>37 (10.7)</td>
<td>47 (13.6)</td>
</tr>
<tr>
<td>Autumn</td>
<td>161</td>
<td>71 (44.1)</td>
<td>45 (28.0)</td>
<td>26 (16.1)</td>
</tr>
<tr>
<td>Total</td>
<td>1439</td>
<td>406 (28.2)</td>
<td>247 (17.2)</td>
<td>171 (11.9)</td>
</tr>
</tbody>
</table>

var. grubii in the present study was higher in the spring (14.2%) than during the rainy season (10.7%), the difference was not statistically significant (P = 0.055). Our results of low prevalence of C. gattii in decayed wood during winter are similar to those of Granados and Castañeda [7] who did not find any C. gattii in the decayed wood of tree trunk hollows. Furthermore, they found only a low population density (cfu/g) of the pathogen in the bark of trees investigated from Bogotá, Colombia, during the winter months of January and February. However, its prevalence was high during April and May. Subsequently, Granados and Castañeda reported a retrospective study [8] in which they analyzed the relationship between the occurrence of various serotypes of the C. neoformans species complex in tree samples and the climatic conditions recorded during samplings in four cities of Colombia, between 1992 and 2004. Their investigations using logistic regression and lagged Pearson correlations indicated that environmental conditions, mainly humidity, temperature and solar radiation variably affected the occurrence of serotypes A, B and C. It was also noted that serotype A (C. neoformans var. grubii) was more thermotolerant and hygrophobic than serotype B. Our data on the prevalence of serotypes A and B during the summer and rainy seasons provide further support for their hypothesis (Table 1).

In an environmental survey in British Columbia, Canada and the Pacific Northwest of the USA, Kidd et al. [9] identified significantly higher concentrations of C. gattii cfu in the air during the warm, dry and low relative humidity summer months (May to August) than during the cold, wet and high relative humidity months. Interestingly, this pattern of seasonal prevalence of C. gattii in the air is similar to what we have observed in our wood sampling. However, contrary to our results, they did not find any seasonal pattern in the prevalence of C. gattii in their investigations of decayed wood, soil and water samples. This partial divergence in results may be attributed to various geoclimatic differences between the geographic regions.

Fig. 1  Seasonal variations in isolation frequencies of Cryptococcus neoformans var. grubii and Cryptococcus gattii in decayed tree trunk wood in north-western India.

Fig. 2  Variations in mean temperature, relative humidity and rainfall during various seasons in north-western India.
investigated. Another possible reason for the differences may be that the C. gattii population investigated by them belonged to a newly emerged molecular type, VGIIa, and not to VGI which encompasses our C. gattii isolates.

As far as we are aware, this is the first study on seasonal variations of the prevalence of C. neoformans var. grubii and C. gattii in decayed wood of living trees from India. It would be extremely interesting to correlate these results with the prevalence of cryptococcosis in patients in the same region in order to determine their inter-relationship. Unfortunately, no such clinical data are available in India at present. However, such comparisons have been done in Thailand and the USA [10,11]. In the Thailand study, 793 cases of cryptococcosis in AIDS patients admitted to Chiang Mai University Hospital were analyzed regarding their prevalence in the dry and rainy seasons but there was no significant difference. In contrast, the USA study found a significantly higher prevalence of cryptococcosis in the fall and winter months than in the spring and summer. A comprehensive prospective study including both environmental and clinical data would be needed to determine the potential health impact of seasonal variations in the prevalence of C. neoformans var. grubii and C. gattii in decayed wood of various trees in climatically divergent regions of India.

Acknowledgments

This work was carried out in part with financial assistance from the Department of Science and Technology, Government of India. Acknowledgment is made to the Indian National Science Academy, New Delhi, for the award of an Honorary Scientist position to H. S. R., and to Dr M. Rahman for assistance with statistical analysis of the data.

Declaration of interest: The authors report no conflicts of interest, and they alone are responsible for the content and writing of the paper.

This paper was first published online on Early Online on 24 September 2010.

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