The emergence of *Cryptococcus gattii* VGII as a super killer?

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Members of the *Cryptococcus neoformans (C.n.)* species complex are basidiomycetous yeast (Figure 1 [A–C]), which cause the second most common life-threatening invasive fungal disease (cryptococcosis) in humans and animals worldwide. The *C.n.* species complex has spawned the recent global emergence of highly virulent strains and is comprised of two closely related species.

The first species, *C. neoformans,* includes two varieties – *C.n. var. grubii* (serotype A, genotypes VNI/AFLP1 and VNII/AFLP1A), the major fungal pathogen in patients with AIDS, and *C.n. var. neoformans* (serotype D, genotype VNIV/AFLP2) as well as an AD hybrid (genotype VNIII/AFLP3) – and is an opportunistic pathogen that typically causes disseminated cryptococcosis in hosts with normal or impaired immunity.

The second species, *C. gattii* (serotypes B and C, genotypes VGI/AFLP4, VGII/AFLP6, VGIII/AFLP5 and VGIV/AFLP7) is a primary pathogen that mainly affects patients with normal immunity [1]. It is also a pathogen of koalas and domestic animals. Infections, are assumed to be acquired via inhalation of infectious propagules, desiccated yeast cells (blastoconidia) or basidiospores, from environmental niches.

In 1999, novel genotypes within the *C. gattii* molecular type VGII emerged on Vancouver Island, British Columbia, Canada, causing a fast spread of the outbreak strains among residents, visitors, and domestic and wild animals [2]. The genotype rapidly became hyperendemic and is currently spreading extensively over the British Columbia lower mainland [3]. The incidence of human cryptococcosis on Vancouver Island escalated from one or two cases a year to 8.5/10 [6] population in 1999, rising to 36/10 [6] in 2005. This infection rate is markedly higher than that seen in *C. gattii* endemic areas in Australia's Northern Territory (18.8/10^6/year). More than eighty human cases in immunocompetent people and more than 300 animal infections in cats, dogs, ferrets, horses and porpoises were reported and led to four human and >100 animal deaths.

This outbreak has sparked intense international research efforts to investigate the origin and spread of the outbreak strain. As a result of these investigations, a drastic shift in the ecological niche and geographic distribution of *C. gattii* was detected, resulting in its adaptation to temperate climate from its original tropical and subtropical habitats [4]. Environmental sampling on Vancouver Island has also revealed a new association with ten different native tree species, including Douglas-fir, alder, maple and Garry oak; in the absence of eucalypts, which were until then the only recognised environmental source. Molecular epidemiological studies using PCR-fingerprinting and AFLP analysis have shown, that two VGII subtypes where responsible for the outbreak: VGIIa/AFLP6a and VGIIb/AFLP6b [4]. Virulence studies in rats and mice revealed that the major outbreak genotype VGIIa is highly virulent, where the minor outbreak genotype VGIIb is less virulent [5].

Multilocus sequence analysis has shown that the minor less virulent outbreak strain (genotype VGIIb) is closely related to strains that have recently caused human/animal infections in the Northern Territory (specifically in Arnhem Land), New South Wales and Western Australia [5,7]. Fraser *et al* have discussed the possibility that the less virulent minor Vancouver Island outbreak genotype may have originated in Australia and was imported together with eucalypts for reforestation of California, USA [5]. The authors suggested that the major high virulent outbreak strain was possibly the result of same sex mating with an unknown mating partner [5] due to the fact that only α mating type strains have been isolated on Vancouver Island [4]. A genetically identical strain had already been isolated in the 1970s in Seattle, Washington State, USA. The outbreak potential of VGII strains has been confirmed in the Perth/Albany region in Western Australia, where at least forty sheep died at the same time, on a single property and an unusually high prevalence of

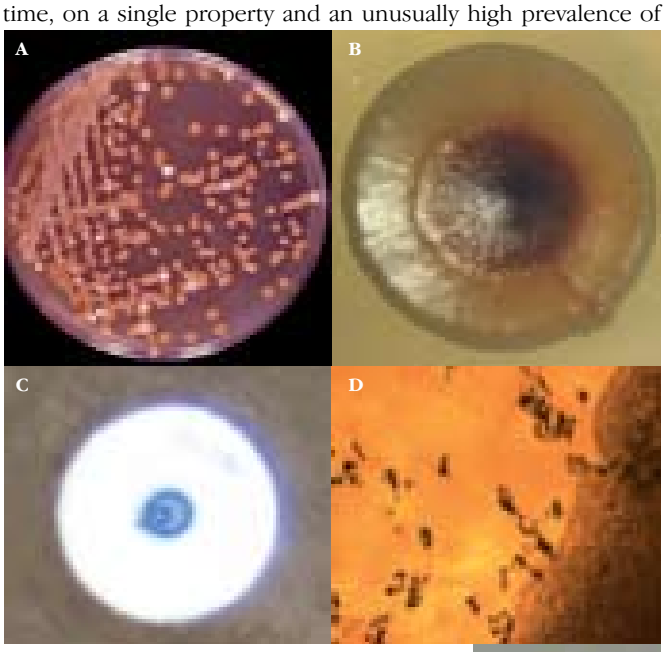

Figure 1. A: *Cryptococcus neoformans* cultured on bird-seed agar.
B: Single colony on SAB agar.
C: Visualisation of the polysaccharide capsule.
D: Filament and spore formation after crosses between strain NIH194 (VGI, MAT**a**) and CDCR265 (VGII, MATα).
E: filaments formed after mating.

VGII infections has been seen in recent years in horses, goats and humans. In Arnhem Land, Northern Territory, VGII infections are hyperendemic, with an incidence of 140/10[6] in the Indigenous population, which is a higher prevalence of cryptococcosis than in any other geographic region.

A subsequent molecular epidemiological study of 160 globally obtained VGII isolates, recovered since 1986, using PCR fingerprinting, AFLP and MLST analysis (8 polymorphic loci: *ACT1, CAP59, IDE, IGS, LAC1, PLB1, SXI1alpha, URA5)* has revealed that strains identical to the high-virulent genotype (VGIIa) have also emerged in Argentina, Brazil and Thailand. Strains identical to the less virulent genotype (VGIIb) are also present in Australia, Brazil and Thailand [9]. VGII isolates from Brazil, Colombia, Greece and Venezuela are closely related to the high- and less-virulent genotypes. The highest genetic variation was found in South American strains. Compared to the Vancouver Island outbreak strains, which are all mating type α, the vast majority of Colombian and some Brazilian VGII isolates are mating type **a** and are less or avirulent. *In vitro* experiments showed that the avirulent Colombian and Brazilian mating type **a** strains mated with the less-virulent VGIIb mating type α strains (Figure 1 [D, E]). The facts a) VGIIa and VGIIb isolates have been isolated as early as 1986 in South America, b) the highest genetic variation was found in South American strains and c) *in vitro* **a**/α mating occurs between South American VGII isolates, indicate that those genotypes may have been present for a long time in the Americas and were spread around the world from there, rather than being a result of a recent recombination event between a less virulent genotype introduced to North America from Australia and an unknown mating partner as suggested by Fraser *et al* [5].

In addition it was shown, that the fungus can colonise wood products, bodies of fresh and salt water, footwear, car wheels, etc [6], opening up several dispersal mechanism allowing for an active or passive spread by humans, birds and marine animals, which could lead to the introduction of high virulent strains to other areas of the world. That is why a close monitoring of the environmental presence of potential virulent genotypes is highly recommended to enable a quick clinical response.

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