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American variants of *Cryptosporidium hominis*: Over-sexed and over here?

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A new study in this issue of *Cell Host & Microbe* from Huang et al. provides important insights into the global epidemiology of human-infectious *Cryptosporidium* and mechanisms leading to the rapid emergence and rise to dominance of new, possibly more virulent, parasite strains.

Human cryptosporidiosis is a major, underestimated contributor to global diarrheal disease through acute and chronic effects.[1] It is caused primarily by anthroponotic *Cryptosporidium hominis* and zoonotic or anthroponotic *Cryptosporidium parvum*. Together, these species are responsible for ~50 thousand deaths annually and have an estimated global burden of 12.8 million disability-adjusted life years.[1]

Due to the lack of effective therapeutics or preventative vaccines, efforts to control *Cryptosporidium* focuses on preventing infections underpinned by a detailed understanding of its epidemiology, population structure, and transmission dynamics.[2,3] Over the last two decades, many have explored these areas using single or multilocus genotyping methods.[4] Most have used the highly variable 60 kDa glycoprotein (*gp60*) gene to subdivide *C. parvum* and *C. hominis* into genotypic families (Ia, Ib, Ic, etc. for *C. hominis* and IIa, IIb, IIc, etc. for *C. parvum*) and subtypes.[4] These studies highlighted a significant richness of *gp60* types, ranging from rare to predominant, geographically stratified to global and, in some cases, associated with varying levels of host adaptation and potential links to differences in infectivity, virulence, or pathogenicity. Notable *C. hominis* variants include IbA10G2, which dominates transmission in many areas of the world, appears associated with increased infectivity and pathogenicity, and was recently proposed as a distinct subspecies, *C. hominis aquapotentis*.[5] Interestingly, as explored in detail by Haung et al.,[6] although *C. hominis* IbA10G2 predominated in the USA prior to 2005, it appears to have been supplanted initially by an Ia strain and, most recently, a newly dominant *C. hominis* IfA12G1R5. However, the study reinforces the concerns around the biological meaning of *gp60*-based variants and whether they effectively represent *Cryptosporidium* populations.

Population genomics studies test these questions and reveal intriguing insights into the biology of *C. parvum* and *C. hominis*.[5,7–10] Huang et al.[6] significantly contribute to this growing body of work. The authors generate many new *C. hominis* genomes, particularly from the USA, filling a major gap in global datasets. They combine these with all publicly available *C. hominis* genomes into a comprehensive, global phylogenomic analysis. The authors show that North American *C. hominis* isolates have cosmopolitan origins, which contrasts with recent indications of geographic clustering and divergence between *C. hominis* from Europe, Africa, or Asia.[5] Although remnants of the proposed European, African, and Asian clades remain visible,[6] it is unclear if this indicates a difference in the importance of regional stratification in old versus new world populations or the need for more detailed sampling in the former.

Huang et al.[6] find that *C. hominis* has a complex population structure consisting of numerous clades. Notably, these are largely inconsistent with *gp60* subtypes. This observation further supports studies highlighting *gp60* as a region of high recombination[5,7–10] and illustrates the need to move beyond this gene as a population marker. Nonetheless, the authors identify monophyly among global *C. hominis* IbA10G2, consistent with its designation as a distinct subspecies, *C. hominis*

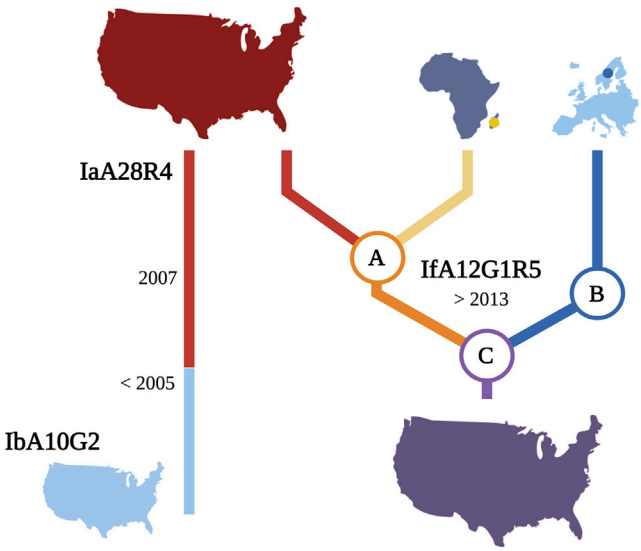

Figure 1. The recombinant origins and rise to dominance of Cryptosporidium hominis variants in the USA revealed through population genomic analyses

Huang et al.[6] use population genomics to retrace the origins, evolution, and mechanisms underpinning the emergence of the predominant *Cryptosporidium hominis* variants in the USA (IfA12G1R5) that have overtaken previously dominant genotypes (e.g., IbA10G2 and Ia28R4). The study reveals evidence of successional introgression through recombination of North America, East African, and European *C. hominis* leading to the rise of a predominant and monophyletic *C. hominis* IfA12G1R5c strain that now dominates US transmission and shows signals of adaptive evolution at gene loci associated with host-parasite interactions. Created with BioRender.com.

aquapotentis.[5] In contrast, the US-predominant *C. hominis* IfA12G1R5 type is divided into three clades (IfA12G1R5a–c; alternatively designated as Pop6, Pop13, and Pop14, respectively).[6]

The *C. hominis* IfA12G1R5a and b are represented by relatively few samples, with most clustering within IfA12G1R5c. Nonetheless, these "minor" IfA12G1R5 clades provide insights into the emergence and success of *C. hominis* IfA12G 1R5. The authors show that *C. hominis* IfA12G1R5a has the most similarity to an "Ia" *gp60*-type population from East Africa.[7] Interestingly, the authors[6] found that IfA12G1R5a acquired genetic material through recombination with several, primarily US-origin, "Ia"-type populations. Noting the current evidence suggesting that IfA12G1R5 emerged in the US or at least was first observed there at a time when Ia types predominated, these findings suggest that an isolate similar to the East African population arrived in the US and recombined with the circulating "Ia" types, leading to the emergence of IfA12G1R5a. In contrast, *C. hominis* IfA12G1R5b may have emerged from something similar to a European off-shoot, although additional global sampling is needed to define this origin more precisely.

The complex origins of IfA12G1R5 in the USA, retraced by these molecular sleuths through whole-genome comparisons, illustrate this important pathogen's ongoing and highly dynamic evolution (Figure 1). New and more transmissible variants evolved from recombination between sympatric strains combined via adaptive introgression and sweeping more transmissible strains at a high frequency. It emphasizes the need for continued high-resolution genetic surveillance as the pathogen is a moving target for those seeking to curtail the disease.

Finally, the authors explore the emergence of the largest IfA12G1R5 clade, IfA12G1R5c, which is the predominant *C. hominis* type in the US.[6] The authors find this clade is a more recent recombinant of IfA12G1R5a and IfA12G1R5b. The authors explored why IfA12G1R5c has become predominant among these subtypes and found evidence of selective sweeps around a number of genes within *C. hominis* IfA12G1R5c relative to other clades. Most were associated with hypothetical proteins and not explored further. However, two genes described as *Cryptosporidium* "mucins," *cgd6_40* and *cgd8_700*, were identified. Various such genes have been identified in recombinant hotspots as being under high selection and likely playing key roles in host interactions in *C. parvum* and *C. hominis* in recent years.[5,7–9] These proteins appear to have a role in host invasion or infection and are intriguing targets for detailed functional studies.

This study fills major gaps in current knowledge of the global population structure of *C. hominis*. In addition, it provides key insights into regional and subspecific differences in the population structure of *C. hominis* and the role of recombination in shaping relationships within and among its lineages. Finally, the work adds important insights into a growing recognition of the importance and complex biology of *Cryptosporidium*'s "mucin" proteins in infection, host adaptation, and virulence, with implications for novel and urgently needed treatments.

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DECLARATION OF INTERESTS

The authors declare no competing interests.

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A selfish bacteriocin dictates pneumococcal domination

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Efficient colonization of new hosts is critical for survival of *Streptococcus pneumoniae*. In this issue of *Cell Host & Microbe*, Aggarwal et al. investigate the population dynamics of this process, demonstrating how a quorum-sensing bacteriocin locus promotes survival of individual clonal lineages, providing a population bottleneck and restricting genetic diversity.

Streptococcus pneumoniae (the pneumococcus) is one of the world's foremost human respiratory pathogens, responsible for >1 million deaths per year, but it is also part of the commensal nasopharyngeal microflora, carried asymptomatically by a significant proportion of the population. Pneumococcal carriage acts as a reservoir for transmission to new hosts, thereby ensuring survival of the species, as well as providing a beachhead for subsequent invasion of deeper tissues to cause disease.[1] Establishment of colonization requires rapid adaptation to the host microenvironment, resistance to host innate defenses, and importantly, competition with other microbes in the host niche. Individual strains of *S. pneumoniae* may differ in their inherent capacity to colonize the nasopharynx and/or in their tendency to cause localized versus invasive disease, with genomic differences impacting a range of virulence-related phenotypes, including capacity to metabolize key nutrients, as well as capacity to trigger host innate defenses.[2,3] In addition to this, Shen et al.[4] have shown that the quorum-sensing competence (*com*) regulon contributes to competition between different *S. pneumoniae* strains in the nasopharynx. Production of the ComC peptide pheromone (CSP) by resident strains that have achieved a critical cell density induces genetic competence as well as a fratricidal mechanism comprising the CibAB bacteriocin and the cell wall hydrolase CbpD. This kills neighboring pneumococci that have not yet achieved competence, thereby excluding "intruder" strains from the niche. As an added bonus, the DNA released from the victims provides a source of donor DNA for genetic transformation of the competent resident, as well as contributing an important extracellular matrix component for biofilm formation.

An elegant study in this issue of *Cell Host & Microbe* by Aggarwal et al.[5] uses a well-established, clinically relevant mouse model to examine the population dynamics of *S. pneumoniae* during the process of colonization. They first constructed a library of 2,764 chromosomally barcoded *S. pneumoniae* 23F isogenic clones, differentiated only by the nucleotide sequence of their respective barcode. Infant mice were then challenged intranasally with the library and colonization levels were followed for 4 weeks, with the proportion of each barcoded clone in nasopharyngeal lavages from individual pups quantitated using next generation sequencing. The overall colonization level increased steadily and reached the maximum capacity after 1 day and remained stable for 4 weeks. Interestingly, at 2–6 h post infection over 50% of the clones were successful in establishing colonization, but the clonal diversity decreased rapidly thereafter. After 1 day, only 28% of the clones in the inoculum were recovered, and by 2 weeks, this had declined to 4.3%. The frequency of the most abundant clone in the population increased from 0.65% in the inoculum to 41.6% at 1 day and 74.5% at 4 weeks post challenge. Remarkably, the most successful clone