

Chlamydiaceae and *Chlamydia*-like organisms (CLOs) in the conjunctiva of children and adults from a trachoma endemic region in Sudan

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Background

Trachoma is the world leading cause of preventable infectious blindness. It is known as a public health problem in 41 countries and is responsible for the visual impairment of about 1.9 million people, of whom 0.450 million are blind. Trachoma is caused by repeated infection of the conjunctiva with *Chlamydia trachomatis*, a bacterium of the phylum *Chlamydiae*. A series of recent investigations have introduced new families to the phylum *Chlamydiae*, known as *Chlamydia*-like organisms (CLOs). Several similarities have been demonstrated between CLOs and *C. trachomatis* concerning their developmental cycle, virulence factors, invading mechanisms and 16S rDNA identity. The main aim of this study was to investigate the frequency of *C. trachomatis* and different families in the order *Chlamydiales* in ocular samples from Sudanese children and adults with trachoma and healthy controls (Figure 1).

Patients & Methods:

Within a case-control study, 96 children (54 cases and 45 controls) and 93 adults (51 cases and 42 controls) were tested using pan-*Chlamydiales* and OmcB Real-Time PCR targeting families of the order *Chlamydiales* and *C. trachomatis*, respectively. Positive samples for pan-*Chlamydiales* Real-Time PCR were subjected to DNA sequencing.



Figure 1: Location of sampling sites: Al-Qadarif region - Sudan. The Map marks the city of Al-Qadarif which is the capital of the state Al-Qadarif in central Sudan.

Results:

The overall prevalence of *Chlamydiales* was 36%. Sequences have been classified into four families including *Chlamydiaceae* (55.6%), *Parachlamydiaceae* (29.6%), *Simkaniaceae* (13%) and *Criblamydiaceae* (1.9%) (Figure 2). Infection with *C. trachomatis* was significantly higher in children (31.5%) compared to adults (0%) with trachoma ($p < 0.0001$) (Figure 3 and 4). 21.5% of adults and 4.2% of children resulted positive for CLOs ($p = 0.0003$) (Figure 3 and 4).

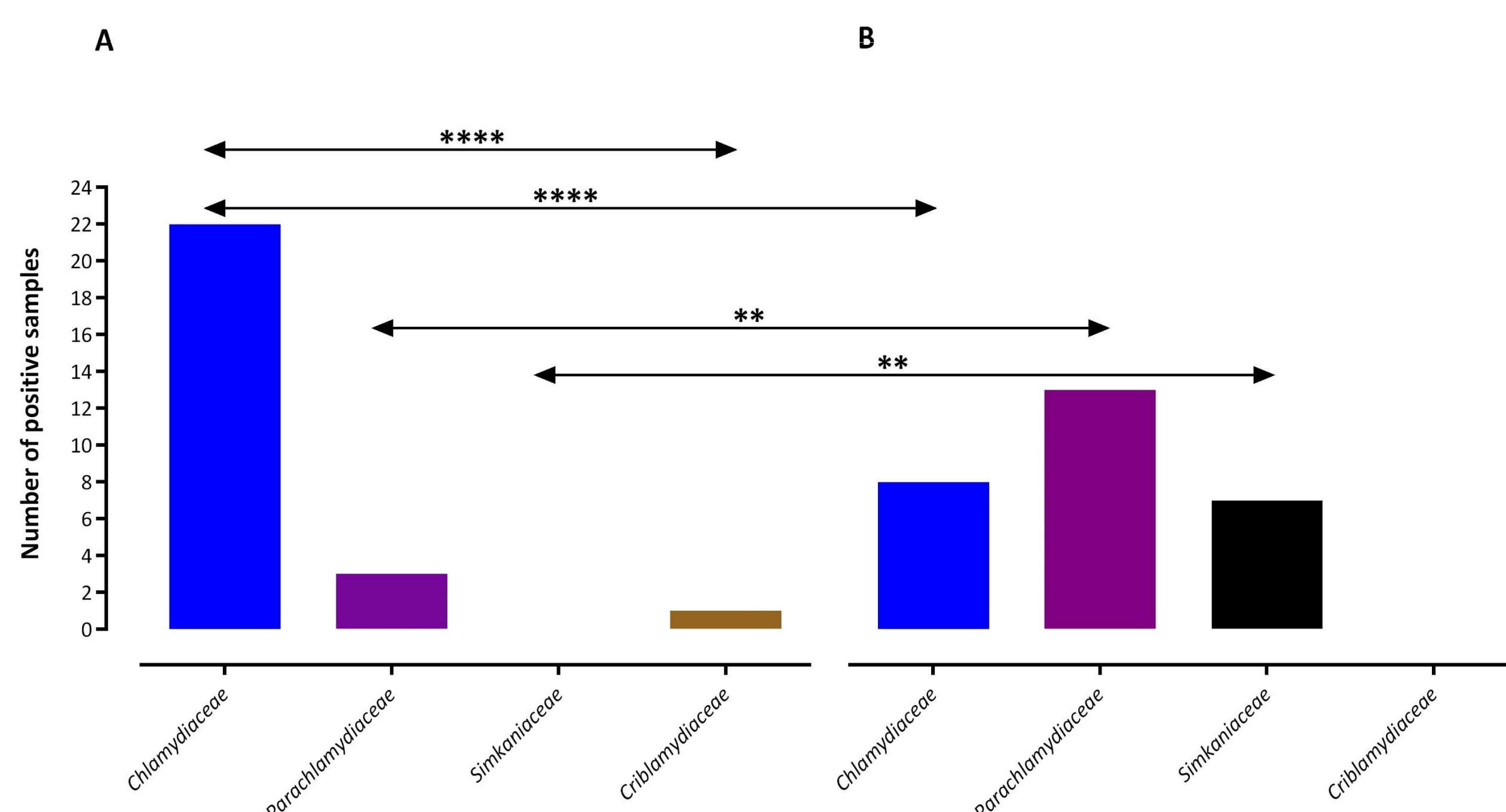


Figure 2: The abundance of classified families in the order *Chlamydiales* in the ocular samples taken from Al Qadarif - Sudan. (A) Distribution of families in the order *Chlamydiales* in sequences achieved from children. (B) Distribution of families in the order *Chlamydiales* in sequences achieved from Adults. The statistical significance is indicated as follows: ** $P < 0.01$, and **** $P < 0.0001$.

Conclusions:

Our findings are consistent with previous results of the central role of *C. trachomatis* in trachoma among children.

This is the first study examining human eyes for the presence of various *Chlamydiales* families. We found an age-dependent distribution of CLOs in human eyes. Differences in the distribution of identified CLOs among trachoma patients and healthy controls cannot suggest a pathogenic role for these bacteria in trachoma. Further studies are needed to understand the impacts of CLOs in pathogenicity and/or protection.

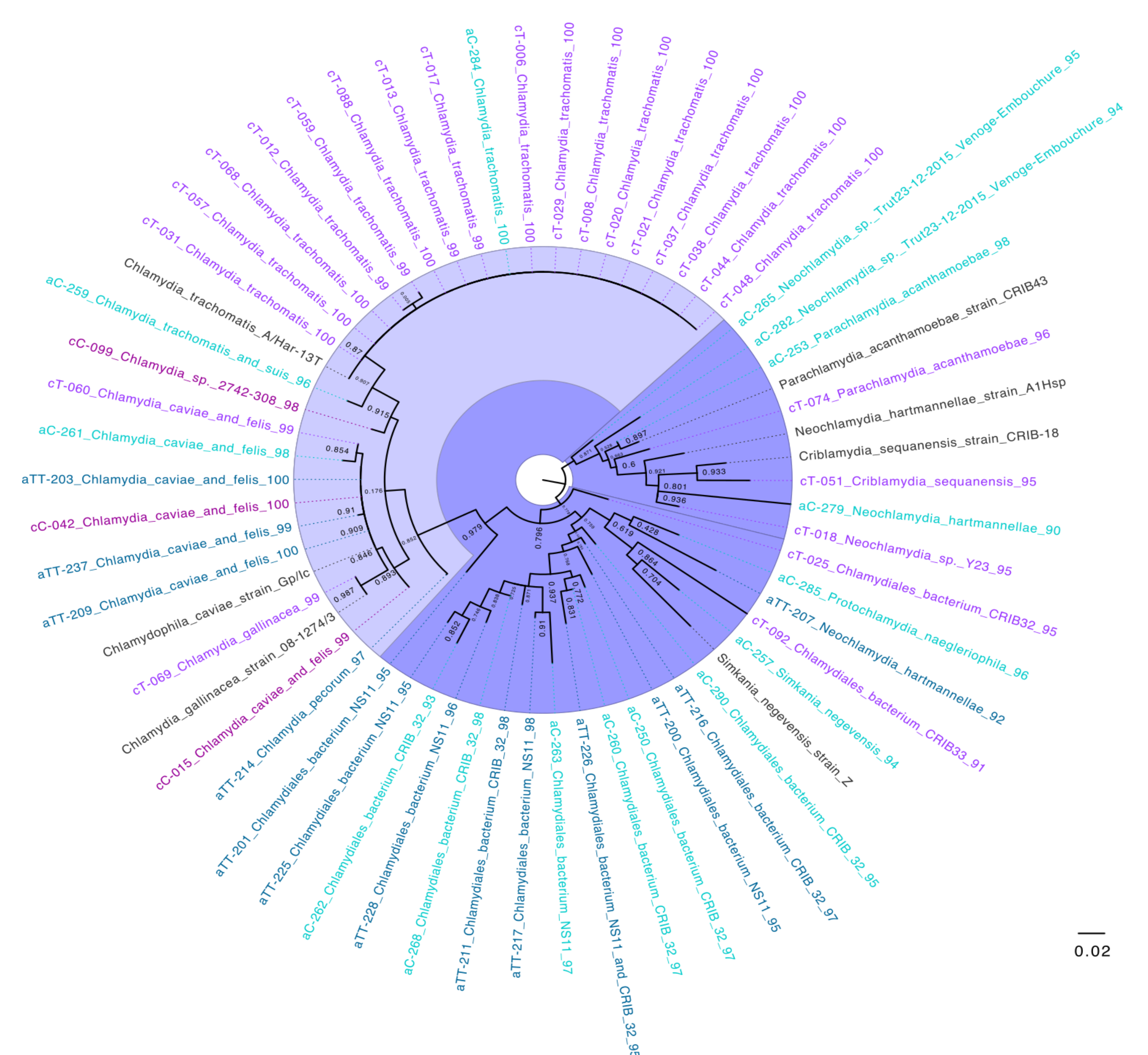


Figure 3: The maximum likelihood phylogenetic tree derived from 16S rRNA sequences. Household IDs of samples are shown on left. Children cases are marked in red, children controls in violet, adults controls in light green, adults cases in dark green and the reference strains corresponding to species for which there was at least one best BLAST hit in black. The best BLAST hit identity (%) for each sample stands on the right side. Uncultured bacteria have been excluded from final BLAST hits by choosing the "Exclude" option in BLAST-n page for "Uncultured/environmental sample sequences".

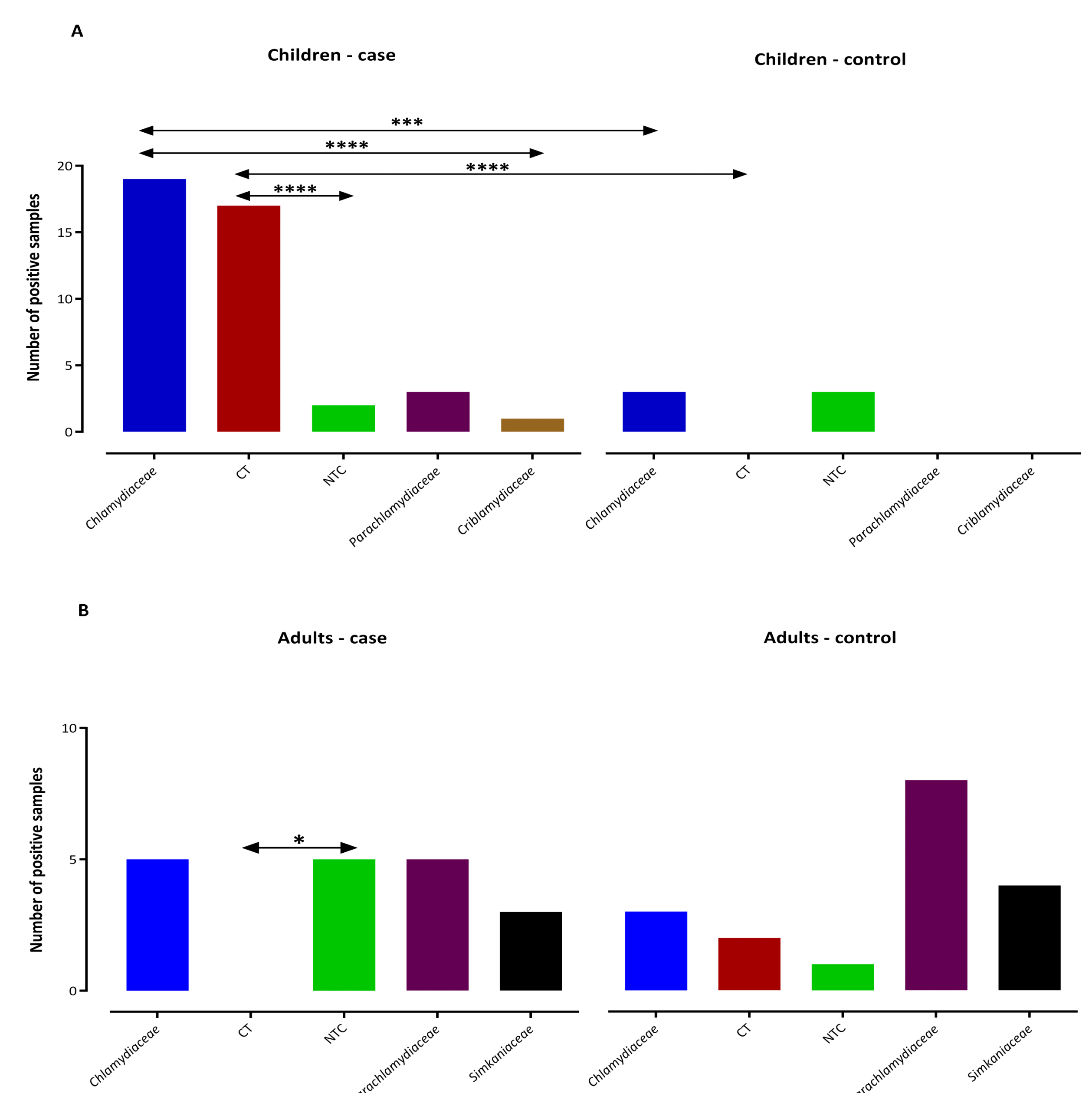


Figure 4: Distribution of families in the order *Chlamydiales*, *C. trachomatis* (CT) and non-trachomat *Chlamydiaceae* (NTC) among children (A) and adults (B). Prevalence of classified sequences *C. trachomatis* in children case group was significantly higher than in children control group. None of the assembled sequences among children and adults were classified as *Simkaniaceae* and *Criblamydiaceae*, respectively. The statistical significance is indicated as follows: * $P < 0.05$, *** $P < 0.001$, and **** $P < 0.0001$.