MULTI LOCUS SEQUENCE TYPING OF *CHLAMYDIALES*: CLONAL GROUPINGS WITHIN *CHLAMYDOPHILA ABORTUS* AND HOST ASSOCIATED GENOTYPES OF *CHLAMYDOPHILA PSITTACI*.

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Introduction

The obligate intracellular growing bacterium *Chlamydia trachomatis* causes diseases like trachoma, urogenital infection and lymphogranuloma venereum with severe morbidity. Several serovars and genotypes have been identified, but these could not be linked to clinical disease or outcome. The related *Chlamydophila pneumoniae*, of which no subtypes are recognized, causes respiratory infections worldwide. *Chlamydophila psittaci* which can cause zoonotic pneumonia in humans are usually hosted by birds, while *Chlamydophila abortus* causes abortion and fetal death in mammals, including humans and is hosted by goats, sheeps and their relatives. We developed a multi locus sequence typing (MLST) scheme based on the partial sequences of seven housekeeping genes to understand the population genetic structure of *Chlamydiales* and the diversity of these species and to evaluate the association between genotype and disease.

Methods

Strains

A collection of 26 strains of *C. trachomatis* of different serovars and clinical presentation and 18 strains of *C. pneumoniae* were included in the study. In addition, 29 *C. psittaci* strains isolated from different bird species and mammals from different geographic locations and 16 *C. abortus* isolated from goats and sheep from different geographic locations were included. For comparison, sequences of *C. caviae*, *C. felis*, *C. pecorum* (*Chlamydophila*), and *C. muridarum* (*Chlamydia*) were also included.

DNA, genes, PCR products and sequences

DNA extraction, PCR protocols and DNA sequencing were performed as previously described (1).

Phylogenetic and other analyses

Sequences of fragments from seven housekeeping genes (*enoA*, *fumC*, *gatA*, *gidA*, *hemN*, *hlfX*, *oppA*) were analysed as described before (1). Allel numbers and genotypes were identified at http://pubmlst.org/chlamydiales/. A distance matrix in Nexus format was generated from the set of concatenated allele sequences using MEGA version 4.0.2 (2), which was then used for phylogenetic analyses in SplitsTree 4.0 (3), generating an UPGMA tree.

Results

Recently, multilocus sequence analysis (MLSA) was introduced to study relatedness of closely related species (4). In this analysis the sequences of multi locus housekeeping fragments are concatenated and used in cluster analysis. Phylogenetic analysis of 38 genotypes among 89 strains of *Chlamydiales* by Neighbour-Joining method of the aligned concatenated sequences of the housekeeping gene fragments resulted in a tree (Fig. 1). comparable to that obtained with 16S rRNA gene and 23S rRNA gene sequences (5). Of note, the distance between *C. abortus* and *C. psittaci* is closer than the distance between the other

species, indicating that *C. psittaci* and *C. abortus* are more related than the other species to each other. Also, the group of *C. abortus* contains one *C. psittaci* strain 84/2334 (ST36) isolated from a Yellow-crowned amazon.
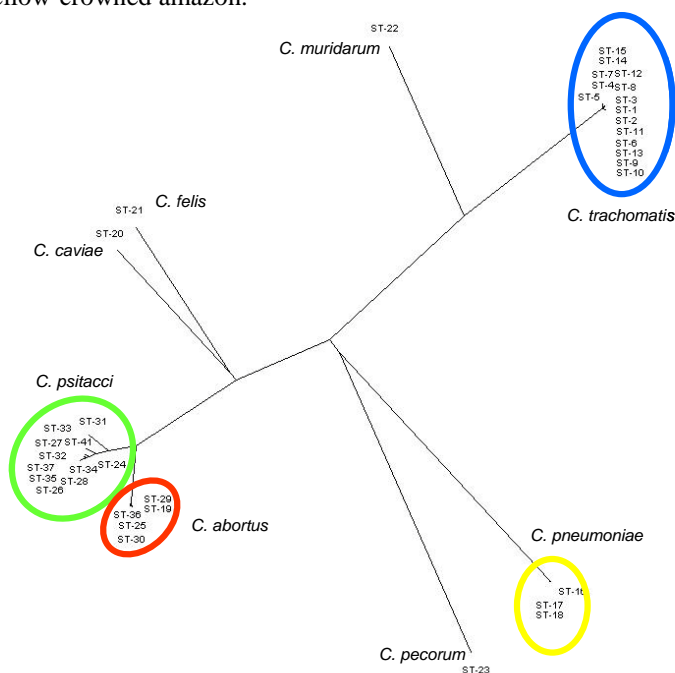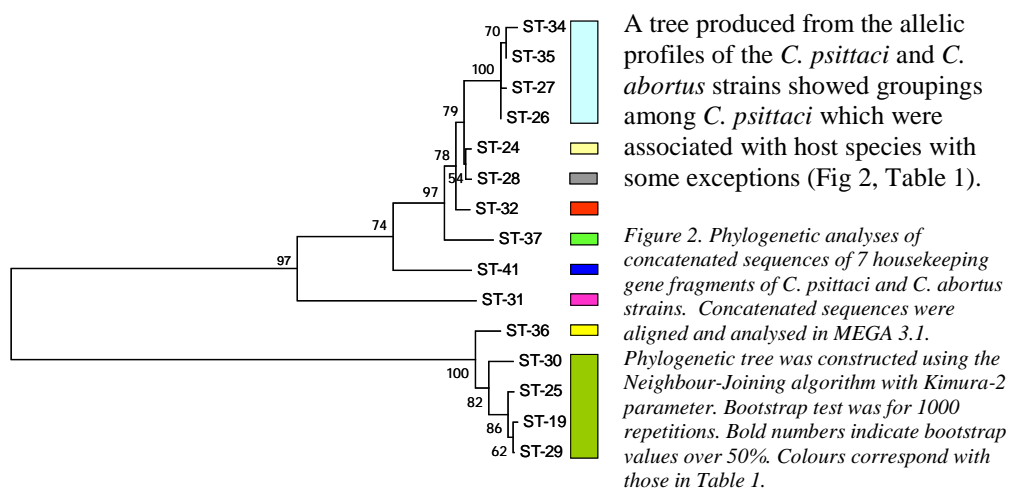

Figure 1. Phylogenetic analyses of concatenated sequences of 7 housekeeping gene fragments of Chlamydiales strains. Concatenated sequences of seven housekeeping gene fragments were aligned and analysed in MEGA 3.1. Phylogenetic tree was constructed using the Neighbour-Joining algorithm with Kimura-2 parameter.

A tree produced from the allelic profiles of the *C. psittaci* and *C. abortus* strains showed groupings among *C. psittaci* which were associated with host species with some exceptions (Fig 2, Table 1).

Figure 2. Phylogenetic analyses of concatenated sequences of 7 housekeeping gene fragments of C. psittaci and C. abortus strains. Concatenated sequences were aligned and analysed in MEGA 3.1. Phylogenetic tree was constructed using the Neighbour-Joining algorithm with Kimura-2 parameter. Bootstrap test was for 1000 repetitions. Bold numbers indicate bootstrap values over 50%. Colours correspond with those in Table 1.

The group of *C. psittaci* strains (ST34, ST35, ST27, and ST26), mainly isolated from pigeons, contains one strain isolated from a ferret and human (ST35). Also, the strains isolated from ducks (all with genotype ST28) are closely related to strains isolated from parrots and

parakeets (all of genotype ST24). Strain WC, divided from these strains with a bootstrap value of 54%.

Table 1. C. psittaci and C. abortus strains according to genotype (ST). Colours correspond to those in Figure 1.

Strain	ST	species	Animal	country
96/1867/30	34	*C. psittaci*	urban Pigeon	Italy
CPMN	35	*C. psittaci*	ferret/human	USA
99/3759/2	35	*C. psittaci*	urban Pigeon	Italy
96/3218	26	*C. psittaci*	urban Pigeon	Italy
98/6098	26	*C. psittaci*	urban Pigeon	Italy
CP3	27	*C. psittaci*	Pigeon	
84/55	24	*C. psittaci*	Budgerigar	Germany
89/1291	24	*C. psittaci*	Budgerigar	Belgium
91/154	24	*C. psittaci*	Budgerigar	Belgium
2000/2675	24	*C. psittaci*	Eastern Rosella	Italy
99/3005	24	*C. psittaci*	Elegant Parrot	Italy
C_psittaci 6BC	24	*C. psittaci*	Parakeet	
VS1	24	*C. psittaci*	Parrot	USA
95/99	24	*C. psittaci*	Peach-faced Lovebird	Italy
2000/332	24	*C. psittaci*	Scarlet Macaw	Italy
91/237	24	*C. psittaci*	Senegal Parakeet	Belgium
90/1551	24	*C. psittaci*		
91/0137	24	*C. psittaci*		
CR9	28	*C. psittaci*	Duck	Europe
humaan E	28	*C. psittaci*	human	
18/290800	28	*C. psittaci*	Pekin Duck	Germany
3/20901	28	*C. psittaci*	Pekin Duck	Germany
4/20901	28	*C. psittaci*	Pekin Duck	Germany
5/20901	28	*C. psittaci*	Pekin Duck	Germany
WC	32	*C. psittaci*	Bovine	
NJ1	37	*C. psittaci*	Turkey	USA
VS225	41	*C. psittaci*	Parakeet	USA
M56	31	*C. psittaci*	Muskrat	
84/2334	36	*C. psittaci*	Yellow-crowned amazon	Germany
LLG	30	*C. abortus*	goat	Greece
POS	30	*C. abortus*	sheep	Greece
1B	25	*C. abortus*	sheep	France
FAG	19	*C. abortus*	goat	Greece
MB	19	*C. abortus*	goat	Greece
VPIG	19	*C. abortus*	goat	Greece
B577	19	*C. abortus*	sheep	USA (ATCC)
A22	19	*C. abortus*	sheep	UK
AB7	19	*C. abortus*	sheep	France
FAS	19	*C. abortus*	sheep	Greece
MA	19	*C. abortus*	sheep	Greece
MD	19	*C. abortus*	sheep	greece
ME	19	*C. abortus*	sheep	Greece
C_abortus	19	*C. abortus*		
MF	19	*C. abortus*		Greece
Krauss-15	29	*C. abortus*	caprine	Tunisia

Clustering is not associated with the geographic origin of the species. Two *C. psittaci* strains are more distantly related to the other *C. psittaci* strains; one isolated from a parakeet and one isolated from a muskrat. Finally, the *C. psittaci* strain 84/2334 isolated from a parrot is closely related to the *C. abortus* strains forming one group.

Discussion

The in this study used MLST scheme was previously applied to analyze clonal groupings among *C. trachomatis* and *C. pneumonia* strains (1). We showed that an UPGMA tree

produced from the allelic profiles and from concatenated allel sequences resulted in three groups of sequence types. The urogenital strains were distributed over two separated groups; one consisted solely of strains with frequent occurring serovars (E, D and F). The LGV strains grouped in a single cluster, which also included *C. trachomatis* B/TW5. Recently, another MLST scheme was described showing clonal groupings among *C. trachomatis* strains with a group consisting exclusively of LGV strains (6). Of note, B/TW5 shares IncA polymorphisms with LGV strains, which were not found among other serovars (7).

Phylogenetic analyses of the concatenated allel sequenes of *C. psittaci* and *C. abortus* strains indicated an association between *C. psittaci* genotype and host species, but not with geographic origin. However, there are exceptions like *C. psittaci* CPMN from a patient found in a cluster with strains isolated from pigeons and *C.psittaci* human E from a patient found in the group with strains from ducks, suggesting host species-crossing by *C. psittaci*. Host species-crossing of *C. abortus* may be indicated by strain 84/2334. Although this strain is annotated as being a *C. psittaci* strain, it groups with all *C. abortus* strains in the MLSA analyses. Previously, this strain was identified as the missing link between *C. psittaci* and *C. abortus* (8). It was shown that 84/2334 has DNA sequences identical to an extrachromosomal plasmid in duck *C. psittaci* strain N352, to rnpB in strain R54 from a brown skua and to the rrn intergenic spacer in parakeet strain Prk/Daruma. Analysis of *ompA* and the rrn spacer revealed progressive diversification of the strains, with 84/2334 resembling what might have been a recent ancestor of *C. abortus*. Our analyses of concatenated sequences of 7 housekeeping alleles which are not under selective pressure of the host show that 84/2334 branches off before all *C. abortus* strains, indicating that this strain differs from *C. abortus* but is more closely related to *C. abortus* than to *C. psittaci* and should therefore be classified as *C. abortus*.

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