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# *Chlamydophila abortus* in a Brown Skua (*Catharacta antarctica lonnbergi*) from a Subantarctic Island

BJÖRN HERRMANN,[1]* RUBAIYAT RAHMAN,[1] SVEN BERGSTRÖM,[2] JONAS BONNEDAHL,[3] AND BJÖRN OLSEN[3,4]

*Section of Virology, Department of Clinical Microbiology, University Hospital, S-751 85 Uppsala,*[1] *Departments of Microbiology*[2] *and Infectious Diseases,*[4] *Umeå University, S-901 87 Umeå, and Department of Infectious Diseases, Kalmar County Hospital, S-381 95 Kalmar,*[3] *Sweden*

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**On Bird Island, South Georgia, a new strain of *Chlamydophila abortus* was detected in one Brown skua out of 37 specimens from six different seabird species. Phylogenetic analysis of the *rnpB* and *omp1* genes indicated the strain to be more closely related to *C. abortus* than to 6BC, the type strain of *Chlamydophila psittaci*.**

The family *Chlamydiaceae* was recently reclassified and now comprises nine separate species (5) that infect a wide variety of animals. *Chlamydophila psittaci* (previously *Chlamydia psittaci*) has been detected in at least 130 bird species (1, 14, 19).

In this study we detected *Chlamydophila abortus* (previously a member of the *Chlamydia psittaci* group) in a Brown skua (*Catharacta antarctica lonnbergi*) on Bird Island (54°1′S, 58°3′W), South Georgian archipelago. The avifauna is abundant, with 24 breeding species of seabirds (17). Shedding organisms from the respiratory tract were obtained by fecal swabs (22) from 37 birds of six different species of subantarctic seabirds. Transportation from Bird Island occurred irregularly; swabs were therefore stored for 3 weeks at −20°C in 0.2 M sucrose-phosphate-buffered saline before being transported to the laboratory. Since culture of *C. psittaci* requires a biosafety lab of class 3, isolation was not attempted.

DNA was extracted from feces by using a QIAamp tissue kit (Qiagen, Hilden, Germany), and the *rnpB* and *omp1* genes were amplified by PCR. The *rnpB* gene encodes the catalytically active RNA subunit of RNase P and is present in all prokaryotic cells and therefore useful for taxonomic analysis (10). For PCR amplification and subsequent sequencing of the *Chlamydophila* sp. obtained from specimen R54 (designated strain R54) from a Brown skua, the primer pair JB1 and JB2 was used (10).

The chlamydial outer membrane protein encoded by *omp1* shows variation between species and strains (8, 11), and the gene was used for characterization of strain R54. For amplification of a 1,032-bp gene segment, a seminested PCR method was used according to the work of Kaltenboeck et al. (12) with minor modifications. The primers in the first step were 9CTROMP (5′GCTCTGCCTGTGGGGAATCCTGCTGAACC3′) and CHOMP371 [5′TTAGAAIC(GT)GAATTGIGC(AG)TTIA(TC)GTGIGCIGC3′], and in the second step the upstream primer was replaced by 29CTROMP (5′GGAGATCCTTGCGATCCTTG3′). The resulting PCR products were sequenced by using terminator-labeled cycle sequencing chemistry and sequence primers, including 29CTROMP, 191CHOMP (5′GCIYTITGGGARTGYGGITGYGCIAC3′), CTR215 [5′TCTTCGA(C/T)TTT(A/T)GGTTTAGATTGA3′], and CHOMP371. Sequence reactions were analyzed on a 310 Genetic Analyzer (PE Biosystems, Norwalk, Conn.).

Sequence alignment was based on a previous analysis (10) and use of the CLUSTAL W multiple alignment program (21). Phylogenetic analysis of the calculated distance matrix was done by using the neighbor-joining program, as previously described (10), and the obtained tree was displayed by using TREEVIEW (15).

In 37 samples from seabirds, one case of chlamydial infection (R54) was detected. The *rnpB* nucleotide sequence of the R54 strain showed a similarity of 97.7% (343 of 351 positions, primer sequences excluded) to a sequence found in nine *C. psittaci* strains, including serovars A to F (10). All eight discrepant nucleotide positions in the 396-bp-long gene product were located in the variable regions of the *rnpB* gene. Interestingly, the *rnpB* gene in strain R54 showed highest similarity (99.2%) to the *C. abortus rnpB* gene when it was compared with all nine species in the *Chlamydiacae* family. The previously determined *rnpB* sequences were identical in eight *C. abortus* strains of bovine, ovine, or caprine origin, but none were derived from birds. Thus, our data from the *rnpB* gene demonstrate that the R54 strain is more closely related to *C. abortus* than to *C. psittaci*.

The striking similarity between R54 and *C. abortus* was supported by analysis of the partially determined sequence (979 bp) of the *omp1* gene in R54. Comparison with previously described nucleotide sequences showed highest similarity (90.7 to 90.9%) to four *C. abortus* strains inducing ovine (B577$^T$ [13], S26/3 [9]) or bovine (BA1 [7]) abortion or enteritis in cattle (LW508 [13]). The sequences were almost identical (90.1%) when R54 was compared to an avian *C. psittaci* type C strain, which is reported to have greater homology to abortion-inducing strains of *C. psittaci* (now *C. abortus*) than to avian *C. psittaci* group A or E strains (see National Center for Biotechnology Information [www3.ncbi.nlm.nih.gov/9 July 1999] accession no. L25436). Several isolated *C. psittaci* strains of porcine origin showed even greater similarity (88.3%) to R54 than to other avian strains of serovar A (type strain 6BC, 81.4% similarity [4]) and serovar D (strain 92-1293, 85.8% similarity [23]). Thus, although serotyping was based on the major outer membrane protein, the immunological reactivity pattern was not directly correlated to the sequence similarity of the *omp1* gene. Serotyping may be used to differentiate strains, but does not fully reflect taxonomic relationships.

Based on the recent reclassification of members of the *Chlamydiaceae* (5), R54 may be classified as *C. abortus*. Phy-

* Corresponding author. Mailing address: Section of Virology, Department of Clinical Microbiology, University Hospital, S-751 85 Uppsala, Sweden. Phone: 46 18 663952. Fax: 46 18 559157. E-mail: bjorn.herrmann@medsci.uu.se.

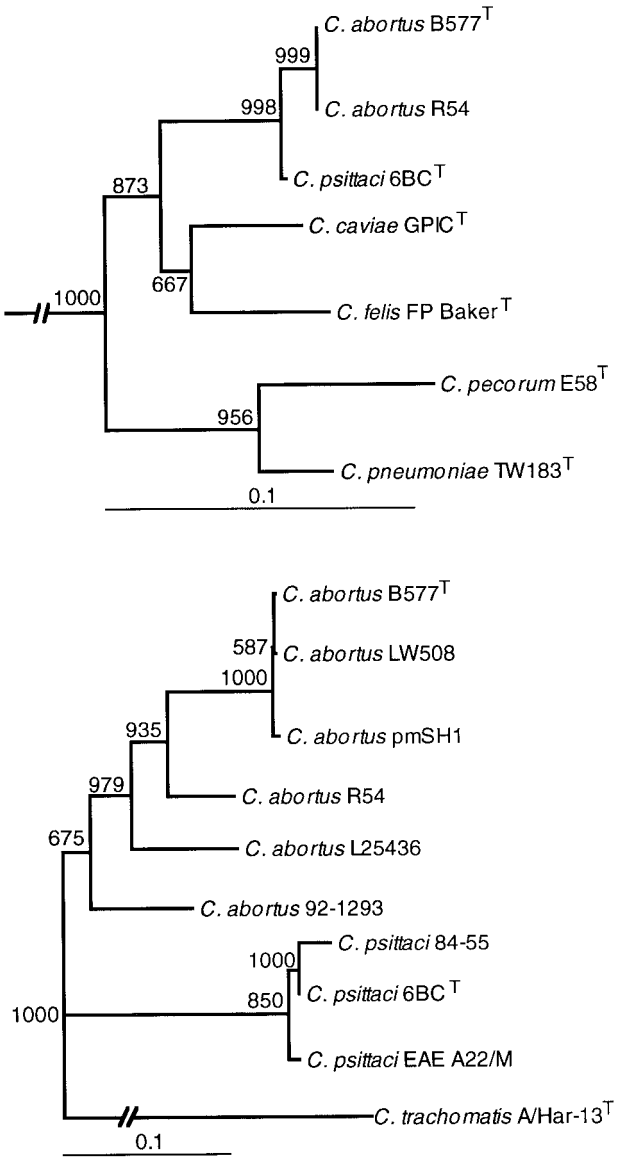


FIG. 1. Neighbor-joining trees based on *rnpB* (upper), showing relationships between the avian strain R54 and type strains of *Chlamydophila* species, and *omp1* (lower), showing relationships among strain R54 and strains of *Chlamydophila* species. Both trees were outgrouped to *Chlamydia trachomatis* strain A/Har-13$^T$ (indicated with broken line in the upper tree). The denoted bootstrap values were obtained from 1,000 resamplings of each data set. The bar indicates 0.1 substitutions per nucleotide.

logenetic analysis by a neighbor-joining program of both the *rnpB* and the *omp1* gene indicated that strain R54 is genetically distinct from *C. abortus* and is still more separated from *C. psittaci* (Fig. 1). Further analysis of other genes, such as the 16S RNA (16), the 23S RNA, and the ribosomal intergenic spacer (3) genes might reveal if congruent evolution has occurred and lead to reclassification of some avian *Chlamydophila* strains.

Certain avian *C. psittaci* strains have been reported to show similarity to ovine abortion-inducing strains in restriction enzyme analysis (6) and in the *omp1* sequence (20); likewise, an avian serovar B was reported from a case of bovine abortion (2). However, the risk of laboratory contamination between avian and abortion-inducing *C. psittaci* strains has been suggested as a possible explanation for the exceptional cases of similarity between strains of different host origins (9, 18). Since both the *rnpB* and the *omp1* sequences of our R54 strain are unique, yet similar to sequences in abortion-inducing strains, it is evident that some avian strains are more similar to abortion-inducing strains than to other avian strains. Further investigations are needed to demonstrate if *C. abortus*-like strains in birds cause clinical manifestations that are different from infections with typical *C. psittaci* strains.

In conclusion, phylogenetic analysis indicated that avian strain R54 is genetically more closely related to *C. abortus* than to the type strain of *C. psittaci*. The impact of *Chlamydophila* infection on the Antarctic fauna must be one of the aims of further studies.

**Nucleotide sequence accession numbers.** Sequences obtained from the R54 specimen were sent to GenBank with the accession numbers AJ243523 for *rnpB* and AJ243525 for *omp1*.

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## ADDENDUM IN PROOF

In an analysis of *ompA* and rRNA sequences by R. Bush and K. D. E. Everett (personal communication) *C. abortus* appears to be evolving away from a cluster of R54-like strains, rather than as a sister clade to *C. psittaci*. Therefore, both ecological data and DNA sequence data must be considered for species identification of a new chlamydial strain. Strain R54 groups ecologically with *C. psittaci* because it was isolated from birds and because there is no evidence that it targets placenta or causes abortion in mammals.

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