Utilization of the ompA gene as a molecular marker for *Chlamydia abortus*

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INTRODUCTION

Chlamydia abortus, an obligate intracellular bacterium belonging to the *Chlamydia* genus, has played a crucial role in both medicine and animal health (Opota, 2015). Its notable ability to induce abortion in ruminants, particularly in sheep and goats (Turin, 2022), positions it as a pathogen of significant economic relevance in the livestock industry, causing substantial losses and threatening the sustainability of animal production. Furthermore, *Chlamydia abortus* has implications for human health, with reports of zoonotic infections resulting from contact with infected animals.

The zoonotic potential and the possibility of environmental transmission of *Chlamydia abortus* are linked to the organism's genome type and genetic diversity (Turin, 2022). Specifically, rearrangements in a variable genome region referred to as the "*Plasticity Zone*" (PZ) and in the tryptophan biosynthetic operon appear to have evolved to enhance adaptation to mammalian hosts and persistence (Thompson, 2005). With the advancement of chlamydial genome sequencing projects, it was discovered that chlamydiae utilize a molecular syringe-like apparatus known as the "type 3 secretion system" (T3SS) (Hsia, 1998). This system delivers effector proteins across host cell membranes to establish a replicative vacuole (Stephens, 1998). One of these outer membrane proteins is MOMP, encoded by the ompA gene.

C. abortus infection can result in fetal loss (abortion) in late gestation stages as well as in the birth of stillborn or weak animals (Arif, 2020), given the bacterium's capacity to progressively colonize the placenta, causing damage and affecting the fetus(es) to varying degrees (Buxton, 2002). The economic impact is compounded by reduced meat and wool production, along with costs associated with diagnosis, treatment, and control measures. Humans can develop flu-like symptoms, pneumonia, and ocular infections, and miscarriages can occur in pregnant women (Essig, 2015). Zoonotic infections emphasize the importance of studying these pathogens from both an animal and human perspective.

Thus, comprehending the evolutionary conservation of the ompA gene among the groups it infects or does not infect helps to enhance the complexity of addressing the challenges posed by this intricate pathogen. A deeper analysis of phylogeny, tracing the gene's evolutionary history among different isolates, can offer insights into diversification over time, contributing to a more comprehensive approach in discussions about this complex infection and its ramifications in the realms of health and economics.

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MATERIALS AND METHODS

The biological sequences used in this study were obtained through the retrieval system of the international NCBI Entrez database (Sayers et al., 2009). A total of 28 samples of the Chlamydia abortus ompA gene were randomly selected from various animals and different locations. The nucleotide sequences underwent a merging step to create a multifasta format file, which was utilized for alignment using the MAFFT program v.7.490 (Katoh et al., 2013).

For phylogenetics, the Maximum Likelihood (ML) technique was employed using the iQtree software v.1.6.12 (Nguyen et al., 2015) to infer the phylogenetic tree that best fit the genomic data. The choice of the evolutionary model, TVM+F+G4, was automatically determined by iQtree based on

the information criterion that best suited the data. The selected nucleotide substitution model was F81+F. Additionally, a bootstrap value of 100,000 was input to assess the support of the tree branches.

The interpretation of the phylogenetic tree was performed using the Interactive Tree of Life (iTOL) platform v.4.2 (Letunic et al., 2016).

RESULTS AND DISCUSSION

The phylogenetic analysis of the ompA gene yielded the topology shown in Figure 1, where it is evident that the gene's utility as a molecular marker among groups infected by the same species is limited (Psarrakos et al., 2011), despite the efficiency of genotyping within the *Chlamydia* genus (Klint et al., 2007). The gene does not appear to be conserved across the groups it infects, and even after various attempts at phylogenetic reconstruction, the bootstrap values did not exceed those obtained in our tree.

Samples M73036.1 and L3902.1, found in Bos taurus, are closely related in an evolutionary sense, supported by the bootstrap value. The same applies to most samples found in *Homo sapiens*, where four of them are closely related and have a present bootstrap value, while two samples are more closely related to a sample of unknown origin. The strains of Ovis aries and Capra hircus displayed significant genetic diversity and were present in several non-monophyletic branches, as evident in the tree (Figure 1). Among the samples extracted from unknown animals, some exhibited high bootstrap values in the branches. For instance, OP414763.1 is genetically close to the strain present in *Ovis*. Conversely, OP414761.1 is monophyletically grouped with the strain present in Columba livia.

The polytomy observed in sample JF728988.1, as well as the evolutionary distance of OP414760.1 and GU320570.1, can be attributed to sequence degradation. These three samples have a significantly lower number of base pairs compared to the gene's average length of 900 to 1000 bp.

Overall, the phylogenetic analysis of the ompA gene reveals a lack of clear conservation among the groups it infects, reflecting the complexity of Chlamydia abortus genetic diversity and its potential impact on the utility of the gene as a robust molecular marker.
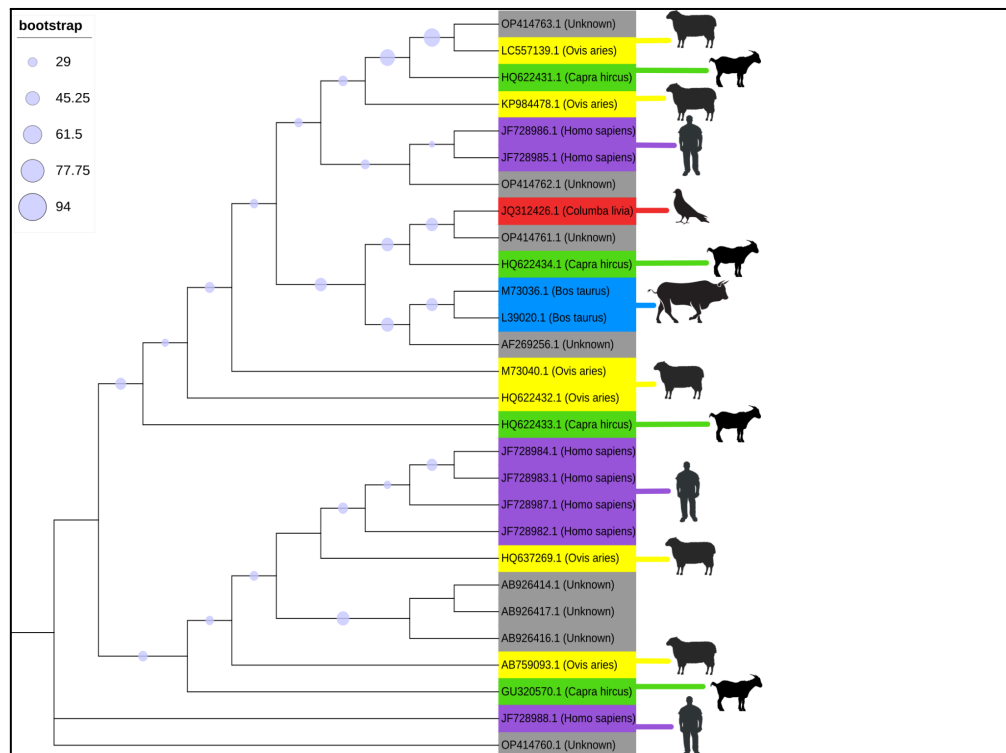
Figure 1. Phylogenetic Tree constructed in iQtree and visualized using iTOL. Taxon colors are related to the organism from which they were collected, C. abortus. In yellow, the species Ovis aries; In purple, Homo sapiens; In green, Capra hircus; In blue, Bos taurus. Gray represents organisms with unknown metadata from the samples. Bootstrap values of the branches are shown in the upper left corner.

CONCLUSIONS

The conclusion drawn from this study is that the ompA gene cannot be effectively utilized as a relevant molecular marker within the biological groups it infects. Several factors contribute to this outcome. The gene's high conservation rate within the species, coupled with the poor quality of sequences deposited in public databases and the limited availability of samples, undermine both evolutionary analyses of this gene and other epidemiologically relevant analyses. In conclusion, this study provides a foundation of information for future research involving this bacterial gene.

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