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Short communication

Molecular characterization of *Chlamydia pneumoniae* in animals and humans from Argentina
Genetic characterization of *Chlamydia pneumoniae*

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ABSTRACT

In this study, genetic diversity of *Chlamydia pneumoniae* was investigated and the relationships between sequences amplified of different sources, clinical conditions and geographical regions of central Argentina were established. Samples amplified were similar to human *C. pneumoniae* patterns and show the high clonality of the population.

Zoonotic infections are a growing threat to global health. The most common atypical pneumonias are caused by zoonotic pathogens, such as *Chlamydia* (*C.*) *psittaci*. *Chlamydia pneumoniae* remains an enigmatic human and animal pathogen. It was discovered in association with acute human respiratory disease (Kutlin et al., 2007; Rattei et al., 2007); now, *C. pneumoniae* is associated with a remarkably wide range of chronic diseases (Cuffini et al., 2006) as well as with a cosmopolitan distribution within the animal kingdom.

C. pneumoniae was initially thought to be an exclusively human pathogen. However, several studies demonstrated that it could also cause infections in a wide variety of animal species (Kutlin et al., 2007; Rattei et al., 2007; Bodetti et al., 2002). Of the animal infections reported, four non-human genotypes have been described (Bodetti et al., 2002).

Since the first isolation of *C. pneumoniae*, all human isolates have been essentially clonal, providing little evolutionary insight. However, several reports showed the close phylogenetic relationship between strains isolated from animals and human strains (Kutlin et al., 2007; Rattei et al., 2007; Myers et al., 2009), whereas other authors have reported genetic dissimilarity among them (Mitchell et al., 2010a; 2010b; Roulis et al., 2015a, 2015b).

C. pneumoniae has been previously reported in reptiles and birds in our region; this finding seen present could represent an important zoonotic reservoir for human disease (Frutos et al., 2014; 2015).

Despite the importance and widespread prevalence of *C. pneumoniae*, few phylogenetic analyses have been conducted to support evolutionary and epidemiological investigations. Herein, we present the data from a study with the aim to deepen into the molecular epidemiology of *C. pneumoniae* obtained from different sources, clinical conditions and geographical regions of central Argentina.

During the 2010–2012 periods, samples were obtained from different sources from Córdoba, Argentina: three conjunctivae swabs from horses, one vaginal swab from captive African Pygmy Hedgehog, and seven pharyngeal swab samples from patients with suspected psittacosis.

The characteristics of *C. pneumoniae* samples are described in Supplementary data 1.

All samples were stored at 4 °C and referred to the Virology Institute, School of Medicine National University of Cordoba, Argentina. Both the Secretary of Environmental of Cordoba province, Argentina and the Ethics Committee of Hospital Clinical University Health center approved the study (CIES 86/79, 2010).

The cotton swabs were placed in 1 ml sucrose-phosphate-glutamate and 200 μl was subjected to DNA extraction using the Accuprep Genomic DNA Extraction Kit (BIONEER, Alameda, CA, USA), according to the manufacturer's instructions.

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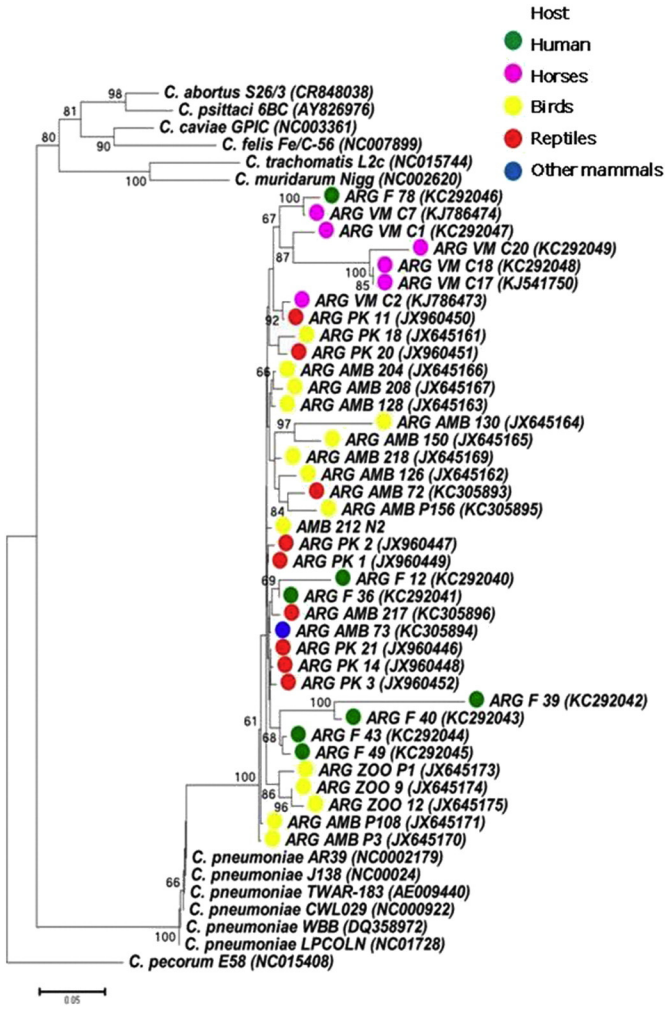

Fig. 1. Neighbour-joining dendrogram based on comparison of concatenated gene *rpoß* and *ompA* of *Chlamydia pneumoniae*. Samples that belong to this study have the prefix ARG AMB and GenBank accession numbers provided. Numbers above branches are bootstrap values as a percentage of 1000 pseudo replicates and only bootstrap values >60% are shown. *C. pecorum* E58 was used as an out-group. Scale bar shows the percentage sequence diversity.

First, 5 μl of DNA extract was used to amplify a final amplicon of 441 pb of the *rpoB* gene, as described by Frutos et al. (2014).

The genotypes of *C. pneumoniae* were analysed using *ompA* gene, as described by Bodetti et al. (2002). The PCR products were purified by gel electrophoresis using the QIAquick Gel Extraction Kit (Qiagen, Valencia, CA, USA) and subjected to direct nucleotide sequencing reaction in both directions by Macrogen, Inc. (Seoul, Korea).

In addition to the new sequences generated as part of this study, a panel of 20 sequences of birds and 9 sequences of reptiles, collected in previous studies, was used (Frutos et al., 2014; 2015). Sequences of *rpoß* and *ompA* genes were submitted to the GenBank (KC292040-KC292049; KJ541750-KJ541758). The sequences of the *rpoß* gene were aligned using the ClustalX program (Conway Institute UCD Dublin, Dublin, Ireland). A dendogram was constructed using the TreeExplorer module of the MEGA program 4 (Tamura et al., 2007) with the neighbour-joining method and *p*-distance parameter. The branch support was evaluated via non-parametric bootstrapping with 1000 pseudo-replicates. The identity matrix was calculated using Distance Matrix tool (IVisTMSA) (Pervez et al., 2015).

Using PCR assays *C. pneumoniae* was detected in birds, reptiles, humans and other mammals from different geographic regions of our province. The phylogenetic analysis showed that all sequences clustered within *C. pneumoniae* (Fig. 1); showing high degree of homology >96% similarity with each other (Table 1). Our sequences showed small differences in human and animal *C. pneumoniae* strains from different geographical regions.

Analyses based on *ompA* gene showed the presence of genotype A in humans and animals, and genotype B in horses (data not shown). The having found the same genotype in humans and animals highlights the possible transmission risk to humans (Mitchell et al., 2010a, 2010b).

Analysis of the *rpoB* gene sequences showed a 10-nucleotide indel CCGTACGAAATA between positions 402–433 nts and one mutation region at position 435–440: AAAGG for TGTGAA was found in all our isolates. These nucleotide changes resulted in amino acid variations. The discrepancies found in these positions could be defined as markers of local strains.

In this study we report the molecular characterization of *C. pneumoniae*, showing very close phylogenetic relationships with a high degree of conservation among samples analysed. This extraordinary genetic conservation and high clonality is in agreement with findings previously reported by Rattei et al. (2007). Nevertheless, Roulis et al. (2015a, 2015b) reported a wide divergence between human and animal strains of *C. pneumoniae*, when they sequenced the whole genome.

Clonality of our sequences may be due to human handling of birds and reptiles and transmitting the infection to animals. This is agreement with Roulis et al. (2013), who postulated the possibility of anthroponosis in the transmission of *C. pneumoniae* infection.

Several limitations of our study need to be considered. We did not perform whole genome analysis because we could not isolate samples in cell cultures, possibly due to the lack of sterile and cooling conditions at the sampling site. Even though a limited amount of sequences was analysed, we found that our samples clustered together. This study reports the first molecular characterization of *C. pneumoniae* in animals and humans in our country. We provide the largest dataset of sequence of *C. pneumoniae* ever reported in Argentina.

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.meegid.2016.06.035.

Table 1
Matrix identity of the samples analysed for the presence of *C. pneumoniae*.

	TWAR-183	AR 39	LPCOLN	WBB	J138	CWL029	ARG VM C1	ARG F12	ARG PK1	ARG AMB 73	ARG AMB 204	ARG AMB 217
TWAR-183	100.00											
AR 39	100.00	100.00										
LPCOLN	99.77	99.77	100.00									
WBB	99.77	99.77	100.00	100.00								
J138	100.00	100.00	99.77	99.77	100.00							
CWL029	100.00	100.00	99.77	99.77	100.00	100.00						
ARG VM C1	91.36	91.36	91.14	91.14	91.36	91.36	100.00					
ARG F12	90.00	90.00	89.77	89.77	90.00	90.00	96.05	100.00				
ARG PK1	94.77	94.77	94.55	94.55	94.77	94.77	96.36	96.36	100.00			
ARG AMB73	94.32	94.32	94.09	94.09	94.32	94.32	95.91	95.91	99.55	100.00		
ARG AMB204	94.09	94.09	93.86	93.86	94.09	94.09	96.59	96.59	99.32	99.32	100.00	
ARG AMB217	93.64	93.64	93.41	93.41	93.64	93.64	95.23	95.23	98.86	98.86	98.18	100.00

Competing interests

The authors declare that they have no competing interests.

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