Journal of Agriculture and Technology Management (JATM)
19(1): 22-25 (January-June 2016)

Short Communication

Phylogenetic Analysis of the 16S rRNA of a *Bartonella* sp. from Cattle in Cebu, Philippines

Adrian P. Ybañez[1,2*] and Rochelle Haidee D. Ybañez[3]

[1]College of Veterinary Medicine at Barili Campus, Cebu Technological University, Barili, Cebu
[2]Department of Research, Gullas College of Medicine, University of the Visayas, Cebu
[3]National Research Center for Protozoan Diseases, Obihiro University of Agriculture and Veterinary Medicine, Obihiro City, Hokkaido, Japan

ABSTRACT

Bartonella sp. is a Gram-negative bacteria that can infect humans and animals, including livestock. In the Philippines, studies on the genus *Bartonella* in livestock have been limited. This study aimed to analyze a 16S rRNA fragment of *Bartonella* sp. previously obtained from cattle in Cebu, Philippines. The aforementioned DNA fragment is believed to be the first molecular evidence of *Bartonella* sp. detected in Philippine livestock. Phylogenetic analyses were performed using neighbor joining, maximum likelihood, and maximum parsimony. The studied sequence was found to be closest to *Bartonella bovis* (99.6%), followed by *Bartonella schoenbuchensis* (99.1%), *Bartonella capreoli* (99.1%) and *Bartonella chomelii* (99.1%). It was least similar to *Bartonella bacilliformis* (97.6). On the other hand, phylogenetic analyses revealed that the obtained sequence consistently clustered with B. *bovis* regardless of the method used. More studies are needed to analyze the detected *Bartonella* sp. using other genes to further assess phylogenetic relationships and confirm the species identification especially that it clustered with the pathogenic B. *bovis*.

KEYWORDS: *Bartonella sp., 16S rRNA, cattle, Cebu*

The bacteria in the genus *Bartonella* are parasites of the red blood cells of wild and domestic mammals (Bermond *et al.*, 2002). They are Gram-negative bacteria from the family *Bartonellaceae* and are facultative intracellular organisms. They are transmitted by vectors, including ticks, fleas, sand flies, and mosquitoes. They are believed to be opportunistic, but it can also be pathogenic (Maillard *et al.*, 2007).

A previous study (Ybañez *et al.*, 2013) reported the first molecular detection of DNA fragments (JQ839016; 450 bp) of a *Bartonella* sp. in a cattle in Cebu, Philippines. These DNA fragments were amplified using primers fD1 and GA1UR. However, comparison with other *Bartonella* spp. and further phylogenetic analyses were not performed. The present study aimed to accomplish these objectives. The obtained sequence was compared to existing DNA database using Basic Local Alignment Sequence Test (BLAST) hosted by the National Center for Biotechnology Information, USA. Percent identities were computed using an EMBOSS pairwise alignment hosted by the European Bio-informatics Institute (http://www.ebi.ac.uk/Tools/emboss/align/index.html).

Phylogenetic analyses were performed by three methods using MEGA 7 (Kumar, Stecher and Tamura, 2016): Neighbor-joining (NJ) and maximum likelihood (ML) and maximum parsimony (Saitou and Nei, 1987; Hasegawa, Kishino and Yano, 1985). All analysis involved 18 nucleotide sequences, a total of 1474 positions in the final dataset and 1000 bootstraps or tree replicates. For the NJ method, the evolutionary distances were computed using the Maximum Composite Likelihood model (Tamura, Nei and and Kumar, 2004). All ambiguous nucleotide positions were removed for each sequence pair.

For the ML method, the analysis was performed based on the Hasegawa-Kishino-Yano model (Hasegawa, Kishino and Yano, 1985). The choice of using the model was based on the results of the search for the best fit model using the same software. Initial trees for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach and then selecting the topology with superior log likelihood value. A discrete Gamma distribution was used to model evolutionary rate differences among sites. For the MP method, branches corresponding to partitions reproduced in less than 50% bootstrap replicates were collapsed. The MP tree was obtained using the Subtree-Pruning-Regrafting (SPR) algorithm (Nei and Kumar, 2000) with search level 1 in which the initial trees were obtained by the random addition of sequences (10 replicates).

The studied sequence was found closest to *Bartonella bovis* (99.6%), followed by *Bartonella schoenbuchensis* (99.1%), *Bartonella capreoli* (NR_025120) (99.1%) and *Bartonella chomelii* (99.1%). It was least similar to *Bartonella bacilliformis* (97.6) (Table 1). The first four *Bartonella*

*corresponding author: dr.adrianpybanez@gmail.com

p-ISSN: 2599-4875 e-ISSN: 2599-4980

spp. mentioned have been detected in ruminants (Dehio *et al.*, 2001; Bermond *et al.*, 2002, Maillard *et al.*, 2004). The identity of the detected Bartonella sp. cannot be confirmed because of the low 16S rRNA sequence identity. For species confirmation using 16S rRNA, at least 99.7% similarity is required (Ybañez *et al.*, 2012).

Table 1. Similarity (%) of the 16S rRNA DNA fragment (450 bp) of a *Bartonella* sp. from Cattle in Cebu (JQ839016) with other Bartonella spp.

Organism	Similarity (%) with *Bartonella* sp. from Cattle in Cebu (JQ839016)
Bartonella bovis (KU859921)	99.6
Bartonella schoenbuchensis (CP019789)	99.1
Bartonella capreoli (NR_025120)	99.1
Bartonella chomelii (JN646644)	99.1
Bartonella henselae (CP020742)	98.9
Bartonella vinsonii AF214558	98.2
Bartonella grahamii CP001562	97.8
Bartonella rattimassiliensis NR_115255	97.8
Bartonella phoceensis NR_115254	97.8
Bartonella bacilliformis CP014012	97.6

The studied sequence was highly similar to B. *bovis*, a known endocarditis agent (Maillard *et al.*, 2007; Erol *et al.*, 2013; Welc-Falęciak and Grono, 2013). This bacteria has been detected in the diseased heart valves of cows by PCR and other molecular methods (Cherry *et al.*, 2009; Marchesi *et al.*, 1998). It can also cause endocarditis in geriatric cows that can be overtly healthy, following chronic bacteremia (Maillard *et al.* (2007).

In the Philippines, the only reported *Bartonella* spp. infections were *B. henselae* and *B. clarridgeiae* in cats (Chomel *et al.*, 1999), and was based on serologic testing. However, the occurrence of *Bartonella* spp. in other animals cannot be ruled out because of the presence of potential vectors. On the other hand, co-infection of *Bartonella* spp. can occur in cattle. Cherry *et al.* (2009) reported four cows that were co-infected with *B. henselae* and *B. bovis*.

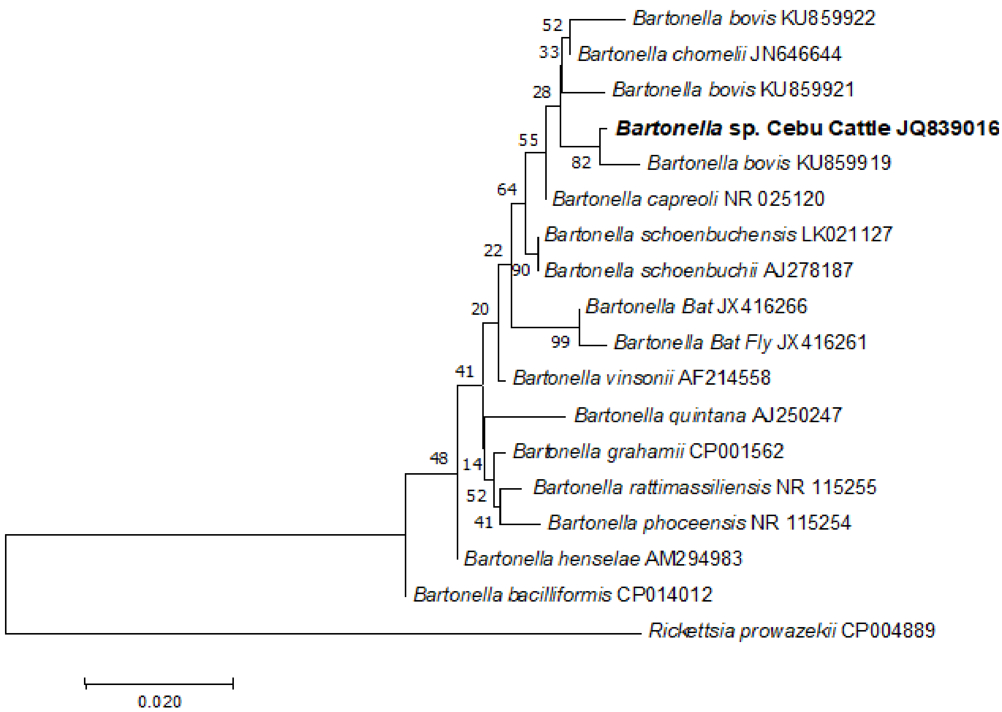

Figure 1. Phylogenetic tree analysis of a *Bartonella* sp. from Cattle in Cebu, Philippines based on the 16S rRNA gene using the Neighbor-Joining method (Saitou and Nei, 1987) by the Maximum Composite Likelihood method (Tamura, Nei, and Kumar, 2004). The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches (Felsenstein, 1985). *Rickettsia prowazekii* was set as the outgroup, and the sequence understudy is set in bold.

p-ISSN: 2599-4875 e-ISSN: 2599-4980

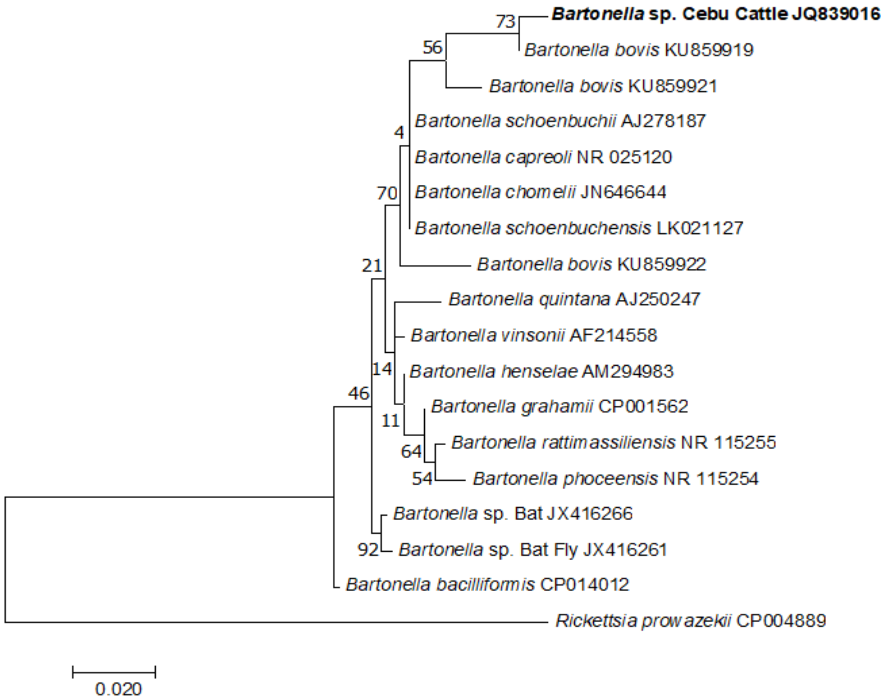

Figure 2. Phylogenetic tree analysis of a *Bartonella* sp. from Cattle in Cebu, Philippines based on the 16S rRNA gene using the Maximum Likelihood method by Hasegawa-Kishino-Yano model (Hasegawa, Kishino and Yano, 1985). The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches (Felsenstein, 1985). *Rickettsia prowazekii* was set as the outgroup, and the sequence understudy is set in bold.

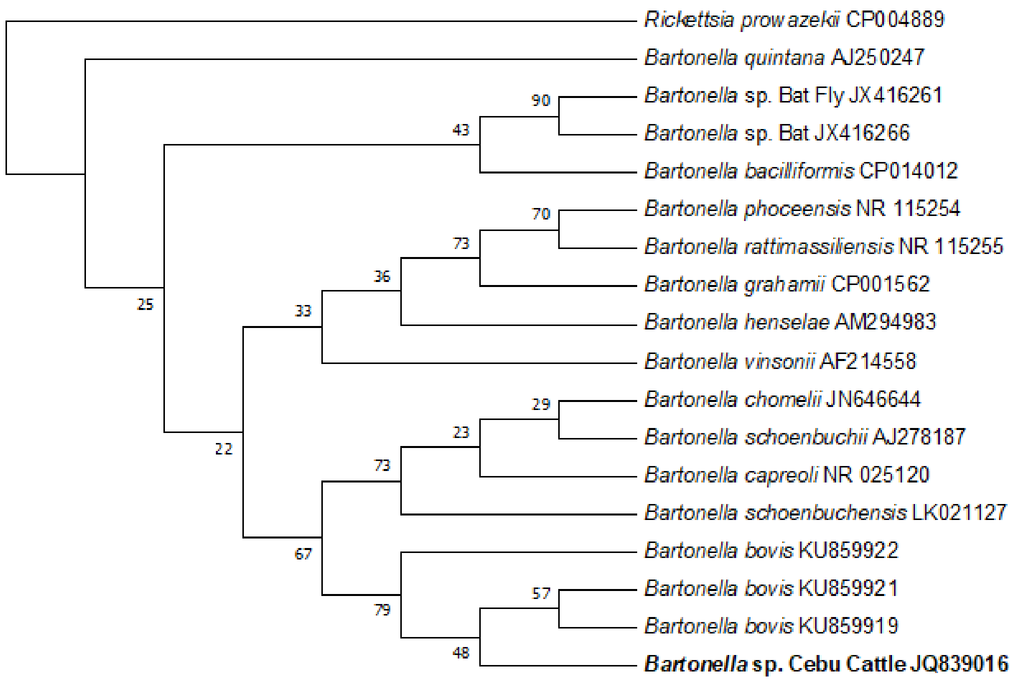

Figure 3. Phylogenetic tree analysis of a *Bartonella* sp. from Cattle in Cebu, Philippines based on the 16S rRNA gene using the Maximum Parsimony (MP) method. Branches corresponding to partitions reproduced in less than 50% bootstrap replicates are collapsed. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches (Felsenstein, 1985). The MP tree was obtained using the Subtree-Pruning-Regrafting (SPR) algorithm (Nei and Kumar, 2000) with search level 1 in which the initial trees were obtained by the random addition of sequences (10 replicates). *Rickettsia prowazekii* was set as the outgroup, and the sequence understudy is set in bold.

p-ISSN: 2599-4875 e-ISSN: 2599-4980

REFERENCES

Andrews, A. H., Williams, B. M. (2004). Endocarditis. In: Andrews AH, editor. Bovine medicine. Oxford:

Blackwell Science Publishing; p. 726–8.

Anderson, B. E. and Neuman, M. A. (1997), Bartonella spp. as emerging human pathogens. Clin Microbiol Rev 10:203–219.

Andrews, A. H. and Williams, B. M. (2004). Endocarditis. In: Andrews AH, editor. Bovine medicine. Oxford: Blackwell Science Publishing; 2004. p. 726–8.

Bai, Y., Malania, L., Castillo, D. A., Moran, D., Boonmar, S., Chanlun, A., ... & Kosoy, M. (2013). Global distribution of Bartonella infections in domestic bovine and characterization of Bartonella bovis strains using multi-locus sequence typing. PLoS One, 8(11), e80894.

Bermond, D., Boulouis, H. J., Heller, R., Van Laere, G., Monteil, H., Chomel, B. B., and Piémont, Y. (2002). Bartonella bovis Bermond *et al.* sp. nov. and Bartonella capreoli sp. nov., isolated from European ruminants. International journal of systematic and evolutionary microbiology, 52(2), 383-390.

Buczinski, S, Tsuka, T, and Tharwat, M. (2012). The diagnostic criteria used in bovine bacterial endocarditis: a meta-analysis of 460 published cases from 1973 to 2011. Vet J 193:349–357.

Cherry, N. A., Maggi, R. G., Cannedy, A. L., and Breitschwerdt, E. B. (2009). PCR detection of Bartonella bovis and Bartonella henselae in the blood of beef cattle. Veterinary microbiology, 135(3), 308-312.

Chomel, B. B., Carlos, E. T., Kasten, R. W., Yamamoto, K., Chang, C. C., Carlos, R. S., Abenes, M.V., and Pajares, C. M. (1999). Bartonella henselae and Bartonella clarridgeiae infection in domestic cats from The Philippines. The American journal of tropical medicine and hygiene, 60(4), 593-597.

Chomel, B. B. and Rolain, J. (2007). Bartonella. In: Manual of clinical microbiology, ed. Murray, PR, 9th ed., pp. 850–861. ASM Press, Herndon, VA.

Dehio, C., Lanz, C., Pohl, R., Behrens, P., Bermond, D., Piemont, Y., Pelz, K., and Sander, A. (2001). Bartonella schoenbuchii sp. nov., isolated from the blood of wild roe deer (2001). Int J Syst Evol Microbiol 51, 1557–1565.

Erol, E., Jackson, C., Bai, Y., Sells, S., Locke, S., and Kosoy, M. (2013). Bartonella bovis isolated from a cow with endocarditis. Journal of veterinary diagnostic investigation, 25(2), 288-290.

Felsenstein, J. (1985). Confidence limits on phylogenies: An approach using the bootstrap. Evolution 39:783-791.

Hasegawa, M., Kishino, H., and Yano, T. (1985). Dating the human-ape split by a molecular clock of mitochondrial DNA. Journal of Molecular Evolution 22:160-174.

Kelly, P. J., Davoust, B., Gomez, J., Raoult, D., and La Scola, B. (2005). Bartonella bovis in cattle in Africa. Veterinary microbiology, 105(2), 155-156.

Kumar, S., Stecher, G., and Tamura, K. (2016). MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets.Molecular Biology and Evolution 33:1870-1874.

Maillard, R., Petit, E., Chomel, B., Lacroux, C., Schelcher, F., Vayssier-Taussat, M., and Boulouis, H. J. (2007). Endocarditis in cattle caused by Bartonella bovis. Emerging infectious diseases, 13(9), 1383.

Maillard, R., Riegel, P., Barrat, F., Bouillin, C., Thibault, D., Gandoin, C., and Piémont, Y. (2004). Bartonella chomelii sp. nov., isolated from French domestic cattle (Bos taurus). International journal of systematic and evolutionary microbiology, 54(1), 215-220.

Marchesi, J. R., Sato, T., Weightman, A. J. (1998). Design and evaluation of useful bacterium-specific PCR primers that amplify genes coding for bacterial 16S rRNA. Appl Environ Microbiol 64:795–799.

Nei, M. and Kumar, S. (2000). Molecular Evolution and Phylogenetics. Oxford University Press, New York.

Saitou, N. and Nei, M. (1987). The neighbor-joining method: A new method for reconstructing phylogenetic trees. Molecular Biology and Evolution 4:406-425.

Tamura, K., Nei, M., and Kumar, S. (2004). Prospects for inferring very large phylogenies by using the neighbor-joining method. Proceedings of the National Academy of Sciences (USA) 101:11030-11035.

Welc-Falęciak, R. and Grono, K. (2013). The first cases of Bartonella bovis infection in cattle from Central Europe. Veterinary microbiology, 162(2), 954-956.

Ybañez, A. P., Matsumoto, K., Kishimoto, T., and Inokuma, H. (2012). Molecular analyses of a potentially novel Anaplasma species closely related to Anaplasma phagocytophilum detected in sika deer (Cervus nippon yesoensis) in Japan. Veterinary microbiology, 157(1), 232-236.

Ybanez, A. P., Sashika, M., and Inokuma, H. (2014). The phylogenetic position of Anaplasma bovis and inferences on the phylogeny of the genus Anaplasma. Journal of Veterinary Medical Science, 76(2), 307-312.

Ybanez, A. P., Sivakumar, T., Ybanez, R. H. D., Ratilla, J. C., Perez, Z. O., Gabotero, S. R., and Inokuma, H. (2013). First molecular characterization of Anaplasma marginale in cattle and Rhipicephalus (Boophilus) microplus ticks in Cebu, Philippines. Journal of Veterinary Medical Science, 75(1), 27-36.

p-ISSN: 2599-4875 e-ISSN: 2599-4980