Investigation of tick-borne bacteria (*Rickettsia* spp., *Anaplasma* spp., *Ehrlichia* spp. and *Borrelia* spp.) in ticks collected from Andean tapirs, cattle and vegetation from a protected area in Ecuador

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Abstract

**Background:** Ixodid ticks play an important role in the transmission and ecology of infectious diseases. Information about the circulation of tick-borne bacteria in ticks is lacking in Ecuador. Our aims were to investigate the tick species that parasitize Andean tapirs and cattle, and those present in the vegetation from the buffer zone of the Antisana Ecological Reserve and Cayambe-Coca National Park (Ecuador), and to investigate the presence of tick-borne bacteria.

**Methods:** Tick species were identified based on morphologic and genetic criteria. Detection of tick-borne bacteria belonging to *Rickettsia*, *Anaplasma*, *Ehrlichia* and *Borrelia* genera was performed by PCRs.

**Results:** Our ticks included 91 *Amblyomma multipunctum*, 4 *Amblyomma* spp., 60 *Rhipicephalus microplus*, 5 *Ixodes* spp. and 1 *Ixodes boliviensis*. A potential *Candidatus Rickettsia* species closest to *Rickettsia monacensis* and *Rickettsia tamurae* (designated *Rickettsia* sp. 12G1) was detected in 3 *R. microplus* (3/57, 5.3%). In addition, *Anaplasma* spp., assigned at least to *Anaplasma phagocytophilum* (or closely related genotypes) and *Anaplasma marginale*, were found in 2 *A. multipunctum* (2/87, 2.3%) and 13 *R. microplus* (13/57, 22.8%).

**Conclusions:** This is the first description of *Rickettsia* sp. in ticks from Ecuador, and the analyses of sequences suggest the presence of a potential novel *Rickettsia* species. Ecuadorian ticks from Andean tapirs, cattle and vegetation belonging to *Amblyomma* and *Rhipicephalus* genera were infected with *Anaplasmataceae*. *Ehrlichia* spp. and *Borrelia burgdorferi* sensu lato were not found in any ticks.

**Keywords:** Ticks, *Amblyomma multipunctum*, *Amblyomma sculpturatum*, *Amblyomma* sp., *Rhipicephalus microplus*, *Ixodes lasallei*, *Ixodes boliviensis*, *Ixodes* sp., *Rickettsia*, *Anaplasma*, *Ehrlichia*, *Borrelia*, Ecuador

Background

Hard ticks (Ixodidae) are arthropods that suck blood from their vertebrate hosts and play an important role in the transmission and ecology of infectious diseases [1]. At least 30 ixodid tick species belonging to *Amblyomma*, *Dermacentor*, *Haemaphysalis*, *Ixodes* and *Rhipicephalus* genera have been documented in Ecuador [2]. These genera are recognized vectors of pathogenic bacteria with medical and veterinary relevance in neotropical regions [3].

In South America, information about the occurrence of tick-borne bacteria in wild mammals, which are frequently exposed to tick-bites, is limited [4,5]. Moreover, several severe and economically important diseases of livestock in tropical regions are caused by tick-borne pathogens (i.e. bovine anaplasmosis caused by *Anaplasma marginale*) that can also infect wildlife species [6].

In Ecuador, the Andean tapir (*Tapirus pinchaque*) is listed as endangered species. Cattle introduction into the Andean tapir refuges (i.e. Cayambe-Coca Ecological Reserve) is negatively affecting tapir populations due to loss of habitat.
this environment, pathogens of domestic animals may threaten health of wild animals and vice versa [7].

It is known that Amblyomma sculpturatum, Amblyomma latepunctatum, Amblyomma multipunctum and Amblyomma ovale tick species infest the Andean tapir in Ecuador [7]. All but A. multipunctum have been found biting humans in South America, and harboring tick-borne microorganisms [8-11]. The knowledge of bacteria transmitted by ticks (potential vectors and reservoirs of microorganisms) in a given area is useful for assessing the risk of infection in humans and animals. Therefore, the aims of our study were: 1- To investigate which tick species parasitize the Andean tapirs and cattle, and those present in the vegetation from the buffer zone of the Antisana Ecological Reserve and Cayambe-Coca National Park in Ecuador, and 2.- To detect and to identify tick-borne bacteria belonging to Rickettsia spp., Anaplasma spp., Ehrlichia spp. and Borrelia spp. genera in the collected tick specimens.

Methods
From May to October 2011 and during February 2012, an investigation was conducted in the buffer zone of the Antisana Ecological Reserve and Cayambe-Coca National Park, Napo Province, Ecuador (Figure 1). This area is located in the basin of the Papallacta River, where ‘The Andean tapir conservation project’ was developing.

Ticks were removed from 6 Andean tapirs, cattle [13 cows (Bos taurus) from 4 farms] and vegetation (10 transects of 2-Km long that were toured twice). Arthropods were kept in tubes with ethanol recording the host/sampling and date, and sent to the Center of Rickettsioses and Arthropod-Borne Diseases, located at the Center of Biomedical Research from La Rioja (CIBIR), Logroño (Spain) for further analysis.

The species were identified based on morphologic criteria following taxonomic keys from the Neotropical region [3,12,13]. DNA was individually extracted using DNeasy Blood & Tissue kit (Qiagen, Hilden, Germany).
| Target gene | Primer name | Primer sequence (5’→3’) | Fragment size (bp) | Tm (°C) | Reference |
|-------------|-------------|--------------------------|--------------------|---------|-----------|
| **Tick species** | 16S rRNA | 16S + 1 | CTGCTCAATGATTTTTAATTGCTGG | 456 | 48 | [14] |
| | 16S – 1 | CCGGTCTGAACTCAGATCAAGTG | 54 |
| | 12S rRNA | T1B | AAACATTAGGATAGATACCCT | 338 | 51 | [15] |
| | T2A | AATGAGACGGACGGGCGATGT | 53 |
| | ITS2 | RIB-4 F | CCATCGAGTGGAAYTGCGAAGGACA | 800 | 55 | [16] |
| | RIB-R | GTGAAATTCTATGCTTAAATTCAGGGGT | |
| **Pan- bacterial** | 16S rRNA | fD1 | AGAGTTTGATCCTGGCTCAG | 1500 | 60 | [17] |
| | rP2 | ACGGCTACTCTGTGTCAGCTT |
| **Rickettsia spp.** | gltA (5’ end) | CS-78 | GCAAGTATCGGTGAGGATGTAAT GCTTCCAATAATACAGGAT | 401 | 48 | [19] |
| | CS-323 | |
| | ompA (semi-nested) | R190.70p | ATGCCGAATATTCTTCAAAA | 631 | 46 | [20] |
| | R190.701n | GTCGGTAAATGGCAGCAGATC | |
| | ompB (nested) | rompB OF | GTAACCAGAATATCGTTTCGTA | 511 | 54 | [22] |
| | rompB OR | GCTTTATAAACACGATAAACCC |
| | rompB SFG IF | GTTATTACGTGCTGCTAACAA | 420 | 56 |
| | rompB SFG/IR | GGTTCGCTCATAACATACAG |
| **Anaplasma/ Ehrlichia spp.** | msp2 | msp3F | CCGACGTTTAGCAAGATAAGAG | 334 | 56 | [26] |
| | mspR | GCCCATAGAACACATCATAAGC |
| | 16S rRNA (nested) | ge3a | CACATCAGAATCGGAAGGATTATTC | 932 | 55 | [27] |
| | ge10r | TTCGTTAAGAAGGATTAAATTCCTCC |
| | ge9f | AACGATATTCTTCTTATAGCTTGT | 546 | 55 |
| | ge2 | GCCAGATTAAAGACGGCTTCCAG |
| | 16S rRNA | EHR 16SD | GGTCYCCAAAGCAAAGTCC | 345 | 55 | [28] |
| | EHR | TAGCAACTCATCAGTTCAG |
| | 16S rRNA | GEP-s | CTGCGCGCAACGCACCATCAGAATGCTCACAAGGGA | 431 | 66 | [29] |
| | GEP | CCTCCTTGGCTACATCAGGTTTACA |
| **Borrelia spp.** | flaB (nested) | Outer 1 | AARAGATGGCGACGTAAAA | 497 | 52 | [30] |
| | Outer 2 | GCATTCTCATTACGAGGATAGT |
| | Inner 1 | ACATTTCTGACAGCAACAGGCTTCTA | 389 | 55 |
| | Inner 2 | GAAGCTGCTGAGCGGAGTCTTGTG |
| | 23SC1 | TAAAGCTGACTAATTACCTA | 380 | 52 | [31] |
Each tick specimen was screened by PCR for both identification of tick species and detection of bacteria including *Rickettsia* spp., *Anaplasma* spp., *Ehrlichia* spp. and *Borrelia burgdorferi* sensu lato (s. l.). Tick species were confirmed by PCR targeting the tick mitochondrial 16S ribosomal RNA (rRNA) [14]. PCR assays for the tick mitochondrial 12S rRNA gene and the tick nuclear 5.8S-28S rRNA intergenic transcribed spacer 2 (ITS2) were also performed for selected samples [15,16]. For the screening of tick-borne bacteria, at least two fragment genes of each genus were tested by PCR assays. The molecular biomarkers selected to identify ticks are among the ones most widely used for the phylogenetics of ticks, being suitable to distinguish between closely related species. Biomarkers for the detection of microorganisms were selected based on our own expertise and according to previously reported usefulness and sensitivity. Target genes, specific primers and PCR conditions are listed in Table 1. Two negative controls, one of them containing water instead of template DNA and the other with template DNA but without primers, as well as positive controls of *Rickettsia slovaca* strain S14ab DNA (obtained from Vero cells inoculated in our facility with a *Derma-centor marginatus* tick from La Rioja, and known to be infected with *R. slovaca*), *Anaplasma phagocytophilum* strain Webster DNA kindly provided by Dr. Raoul (Unité de Recherche sur les Maladies Infectieuses et Tropicales Emergentes, France) and Dr. Dumler (The Johns Hopkins Hospital, USA), or *Borrelia burgdorferi* sensu stricto DNA kindly provided by Dr. Fingerle (German National Reference Centre for Borrelia, Germany) were included in all PCR assays. PCR products were sequenced in both directions. Sequences were compared with those available in the NCBI database using BLAST.

### Results

#### Identification of ticks

A total of 161 ticks (75 removed from Andean tapirs, 66 from cattle and 20 collected over vegetation) were included in the study. Ten specimens (one of each stage and gender in case of adult ticks) were deposited in the Museum of Zoology of Pontificia Universidad Católica from Ecuador.

Morphologically, 84 specimens (12 nymphs, 47 male and 25 female ticks) corresponded to *A. multipunctum*, 4 specimens to *A. sculpturatum* and 7 were classified as *Amblyomma* spp. For all but 4 specimens, the mitochondrial 16S rRNA sequences (409 bp) were identical to the 16S rRNA gene from *A. multipunctum* (GenBank accession no. KC677673), or differed by 0.2-1.7% (1-7 bp) when compared to this species. No 12S rRNA sequence from *A. multipunctum* was available in GenBank. Therefore, ours (from a specimen whose 16S rRNA sequence was identical to *A. multipunctum* KC677673) was deposited in GenBank under no. KM077433. It differed in sequence by 10% when compared to those available, and showed the highest identity (90%) with the 12S rRNA gene from *Amblyomma* sp. (GenBank accession no. AY342251).

For the 4 tick specimens morphologically classified as *A. sculpturatum*, sequences of the 16S rRNA showed maximum identity (90%; 370/410 bp) with *A. multipunctum*, whereas 12S rRNA and ITS2 sequences were closest to *Amblyomma varium* (90.6% identity; 309/341 bp and 93.6% identity; 836/893 bp, respectively). Obtained sequences showed lower percentages of identity when compared to those from *A. sculpturatum*: 87% for 12S rRNA (GenBank accession no. AY342276), and 90% for ITS2 (GenBank accession no. AY619574). Therefore, these 4 ticks were classified as *Amblyomma* spp. and these three fragment genes were deposited in GenBank under nos. KM077434-6.

A total of 60 specimens were morphologically classified as *Rhipicephalus microplus* (formerly, *Boophilus microplus*) (6 nymphs, 16 male and 38 female ticks). In all these cases, the 16S rRNA sequences were identical to the 16S rRNA gene from *R. microplus* (GenBank accession no. EU918187).

According to morphological features, 5 female ticks were classified as *Ixodes lasallei*. The 16S rRNA sequences did not match with those from *I. lasallei* (GenBank accession no. AF549850) but were closest to this tick species (90% identity). Due to this discrepancy, they were classified as *Ixodes* spp. and deposited in GenBank under no. KM077438.

Lastly, one specimen morphologically corresponded to *Ixodes boliviensis*. The 16S rRNA sequences showed the highest identity (94%) with the 16S rRNA gene from *Ixodes* sp. (GenBank accession no. KA702251). It was deposited in GenBank since no sequences for *I. boliviensis* were available (KM077437).

According to morphological and genetic classifications, our ticks included 91 *A. multipunctum*, 4 *Amblyomma*

### Table 1 PCR primer pairs used in this study (Continued)

| Primer Pair | Sequence (5' to 3') | Tm (°C) | Length (bp) |
|-------------|---------------------|--------|-------------|
| 55-23Sintergenic spacer (nested) | ACCATAGACTTATATTCTTGGACG | 45 | 226 |
| 55CB | GAGTAGGTTATGCGAGGG | 50 | 55 |
| 23SN1 | ACCATAGACTTATATTCTTGGACG | 45 | 226 |
| 23SN2 | ACCATAGACTTATATTCTTGGACG | 45 | 226 |

bp: base pairs; Tm: melting temperature; Y = C/T; R = A/G; W = A/T.
spp., 60 *R. microplus*, 5 *Ixodes* spp. and 1 *Ixodes boliviensis* (Table 2).

**Detection and identification of tick-borne bacteria**

Tick-borne bacteria were tested for 151/161 specimens, excluding those deposited in the museum. The presence of rickettsiae was screened by PCR assays targeting 2 fragments of the *gltA* rickettsial gene (1019 and 401 pb, respectively). Positive amplicons were obtained for 3 *R. microplus* (2 male and 1 female specimens) removed from 2 cows from different farms. There were no differences in the sequences of *gltA* for amplicons derived from the DNA of the 3 rickettsial-infected *R. microplus*, and showed maximum identities (99.7% - 99.2%) with *gltA* gene from *Rickettsia monacensis* and *Rickettsia tamarcae* as validated species (Table 3).

Subsequently, fragments of *ompA* (532 bp), *ompB* (420 bp), *sca4* (928 bp), 16S rRNA gene (426 bp and 1500 bp, respectively), and 17 kDa-antigen gene (334 bp) were amplified to classify the *Rickettsia* at the species level. The sequences of *ompA* (also identical each other) were closest to *R. tamarcae* (95.9% identity) and *R. monacensis* (95.7% identity) (Table 3).

For *ompB*, the DNA sequences of the 3 rickettsiae-positive *R. microplus* were identical to each other and showed 99.2% identity with *R. monacensis* and 97.1% identity with *R. tamarcae* (Table 3).

Unfortunately, no amplicons were obtained in PCR assays targeting *sca4* gene. Attempts to sequence the rickettsial 16S rRNA and pan-bacterial 16S rRNA amplicons for the 3 *R. microplus* were inconclusive for *Rickettsia*. Curiously, *A. marginale* was amplified in 1 out of these 3 specimens using pan-bacterial 16S rRNA primers (see below). In addition, the sequences of 17 kDa antigen gene did not match with those available in GenBank.

In 2005, Raoult et al. established the criteria for the taxonomic classification of potential new *Rickettsia* species [32]. They proposed the ‘*Candidatus*’ status for a bacterium not established in pure culture that did not exhibit more than one of the following percentages of nucleotide identity: >99.8, >99.9, >98.8, >99.2, and >99.3 for *rrs* (16S rRNA), *gltA*, *ompA*, *ompB*, and *sca4*, respectively, with a validated *Rickettsia* species. According to our results, only amplicons for the *gltA*, *ompA*, *ompB* and 17KDa were obtained. Therefore, based on the recommended nomenclature [32], a *Candidatus* status could not be assigned to this microorganism. We designated this bacterium as *Rickettsia* sp. 12G1.

The presence of *Anaplasma* spp. was detected in 15 out of 151 samples, including 2 *A. multipunctum* and 13 *R. microplus*. On the one hand, the partial sequences of *msp2* and 16S rRNA gene from *A. multipunctum* (a female tick from Andean tapir and a male tick from vegetation) and 8 *R. microplus* (all female ticks from one cow) were, when available, closest (96.6-100% identity) to *A. phagocytophilum* (Table 4). On the other hand, the 16S rRNA sequences (EHR and GEP regions) for 5 *R. microplus* (3 female and 2 male specimens) removed from 3 cows in two farms, were respectively identical each other, and matched (100% identity) with more than one *Anaplasma* species (assigned to *A. marginale*, *Anaplasma ovis*, *A. phagocytophilum* and *Anaplasma centrale*) for both PCR targets. Maximum identity with

### Table 2 Ticks included in this study

| Host/sampling | Tick species | Nymph | Male | Female | Total number |
|---------------|--------------|-------|------|--------|--------------|
| Andean tapir  | *Amblyomma multipunctum* | 2     | 45   | 24     | 71           |
|               | *Amblyomma* spp. | 4*    | 4    |        | 4            |
| Cattle        | *Rhipicephalus microplus* | 6*    | 16*  | 38*    | 60           |
| *Ixodes* spp. | 5*            | 5     | 5    |        | 5            |
| *Ixodes boliviensis* | 1*       | 1    |
| Vegetation    | *A. multipunctum* | 10*   | 9**  | 1*     | 20           |

*Specimen deposited in the Museum of Zoology of Pontificia Universidad Católica from Ecuador.

![Table 2](https://www.doi.org/10.1186/s13071-015-0888-8)

### Table 3 Maximum identities of rickettsial sequences detected in 3 *Rhipicephalus microplus* from Ecuador with validated *Rickettsia* species

| Gene sequence | % identity with *Rickettsia* spp. (bp) | *R. monacensis* GenBank no. | *R. tamarcae* GenBank no. |
|---------------|--------------------------------------|-----------------------------|---------------------------|
| *gltA* [KF831358] | 99.5 (625/628) | DQ100163 | 99.2 (623/628) | AF394896 |
| *gltA* (5’ end) [KF831359] | 99.7 (349/350) | DQ100163 | 99.4 (348/350) | AF394896 |
| *ompA* [KF831361] | 95.7 (444/464) | DQ100169 | 95.9 (445/464) | DQ103259 |
| *ompB* [KF831360] | 99.2 (379/382) | EF380356 | 97.1 (371/382) | DQ113910 |

bp: base pairs; [ ]: GenBank accession number generated in this study; GenBank no.: GenBank accession number; R.: *Rickettsia*. 

![Table 3](https://www.doi.org/10.1186/s13071-015-0888-8)
| Bacterium (no.) | Host/ sampling | No. and stage of tick species | Gene | msp2 | 16S rRNA | 16S rRNA (EHR) | 16S rRNA (GEP) | Pan-bacterial 16S rRNA |
|----------------|---------------|-------------------------------|------|------|----------|--------------|---------------|---------------------|
|                |               |                               |      |      | Maximum % identity (bp) | GenBank acc. no. | Maximum % identity (bp) | GenBank acc. no. | Maximum % identity (bp) | GenBank acc. no. | Maximum % identity (bp) | GenBank acc. no. | Maximum % identity (bp) | GenBank acc. no. |
| A. phagocytophilum or closely related genotypes (10) | Tapir | 1F A. multipunctum | 99.3 (290/292) | CP000235 | ND | ND | ND | NP |
| | Farm 1, Cow 1 | 3F R. microplus | 96.6-98.3 (280-285/290) | FJ600595 | ND | ND | ND | NP |
| | 2F R. microplus | 97.9 (284/290) | CP000235 | ND | ND | ND | NP |
| | 1F R. microplus | 96.6 (282/292) | AY164493 | 100 (497/497) | JF893938 | ND | ND | NP |
| | 1F R. microplus | 96.5 (278/288) | AY164493 | ND | ND | ND | NP |
| | 1F R. microplus | 96.9 (281/290) | FJ600595 | 100 (497/497) | JF893938 | ND | ND | NP |
| | Vegetation | 1M A. multipunctum | 99.3 (289/291) | AY626255 | ND | ND | ND | NP |
| A. marginale (2) | Farm 1, Cow 2 | 1F R. microplus | ND | ND | 100 (305/305) | CP001079 | JN558818 | DQ648489 | CP001759 | JN558818 | 99.4 (1222/1229) | JQ480818* |
| | Farm 1, Cow 3 | 1F R. microplus | ND | ND | 100 (305/305) | CP001079 | JN558818 | DQ648489 | CP001759 | JN558818 | 99.4 (1222/1229) | JQ480818* |
| Anaplasma spp. (3) | Farm 1, Cow 2 | 1M R. microplus | ND | ND | 100 (305/305) | CP001079 | JN558818 | DQ648489 | CP001759 | JN558818 | 99.4 (1222/1229) | JQ480818* |
| | Farm 1, Cow 3 | 1M R. microplus | ND | ND | 100 (305/305) | CP001079 | JN558818 | DQ648489 | CP001759 | JN558818 | 99.4 (1222/1229) | JQ480818* |
| | Farm 2, Cow 1 | 1F R. microplus | ND | ND | 100 (297/297) | CP001079 | JN558818 | DQ648489 | CP001759 | JN558818 | 99.4 (1222/1229) | JQ480818* |

*Coxiella endosymbiont of Rhipicephalus turanicus isolate DGGE.
A. phagocytophilum: Anaplasma phagocytophilum; A. multipunctum: Amblyomma multipunctum; A. marginale: Anaplasma marginale; R: Rhipicephalus; M: male; F: Female; ND: Not detected; NP: Not performed; CP001079-CP000030: Anaplasma marginale sequences from GenBank; JN558818: Anaplasma ovis sequence from GenBank; DQ648489-EU436153: Anaplasma phagocytophilum sequences from GenBank; CP001759: Anaplasma centrale sequence from GenBank.
validated species of *Ehrlichia* genus did not exceed 95% with any of these 16S rRNA target genes (Table 4). Since these fragment genes were highly conserved for these species, in an attempt to identify the *Anaplasma* species, DNA extracts of these 5 samples were used as templates of pan-bacterial 16S rRNA PCR assays. The sequences corresponding to 2 out of 5 *R. microplus* were identical to each other and homologous (100% identity) to *A. marginale*. In these 2 cases, percentages of identity were 99.6, 99.5 and 97.2% when compared to *A. ovis*, *A. centrale* and human pathogenic *A. phagocytophilum*, respectively. Sequencing results for the 3 remaining ticks matched (99.4% identity) with a *Coxiella* endosymbiont of *Rhipicephalus turanicus* (GenBank accession no. J480818) (Table 4).

Table 5 summarizes the detection rates for *Rickettsia* spp. and *Anaplasma* spp. *Ehrlichia* species were not amplified in any of the 151 ticks analyzed in this study. Lastly, *B. burgdorferi* s.l. was not detected in any ticks when *flaB* gene and 5S-23S rRNA intergenic spacer region were tested by PCR.

**Co-infections**

Out of 18 positive ticks, one of them (5.6%) was found co-infected with 2 bacteria. The co-infection detected was *A. marginale* with *Rickettsia* sp. 12G1 in one *R. microplus* tick collected from a cow.

**GenBank accession numbers**

Sequences obtained in this study have been deposited in the GenBank database under the following accession numbers: KM077433-8 (identification of ticks) and KF831358-62 (rickettsial genes).

**Discussion**

A total of 161 ticks (nymphs or adult specimens) removed from Andean tapirs, cattle and vegetation, and belonging to *Amblyomma*, *Rhipicephalus* and *Ixodes* genera, was included in the present study. These tick genera had been previously reported to occur in Ecuador [3,33,34]. Based on morphological and genetic criteria, arthropods were classified as 91 *A. multipunctum*, 4 *Amblyomma* spp., 60 *R. microplus*, 5 *Ixodes* spp. and 1 *I. boliviensis*. On the one hand, *A. multipunctum* was collected from vegetation and found attached to Andean tapirs. This tick species was originally described from a *Tapirus* sp. in North America, and it has been reported in Venezuela, Colombia and Ecuador [35,36]. Partial sequences of the mitochondrial 16S rRNA gene of *A. multipunctum* specimens from Ecuador had been previously generated [37]. Our group has completed this molecular description with sequences of the 12S rRNA fragment gene (GenBank accession no. KM077433). On the other hand, *R. microplus* and *Ixodes* spp. were removed from cows, as well as one specimen of *I. boliviensis* that was genetically characterized herein using mitochondrial 16S rRNA gene as PCR target (GenBank accession no. KM077437). *R. microplus*, known as the cattle tick, is widely distributed in cattle from tropical regions [3]. This is the first description of *I. boliviensis* in Ecuador, although it has been found in cattle from Costa Rica [38].

As far as we know, this is the first report where ticks from Ecuador were evaluated for the presence of *Rickettsia* spp., *Anaplasma* spp., *Ehrlichia* spp. and *Borrelia* spp.

The circulation of a potential *Candidatus* *Rickettsia* species (designated *Rickettsia* sp. 12G1) in *R. microplus* ticks removed from cattle in Ecuador is reported. According to our data, this novel *Rickettsia* was closest to *R. monacensis* and *R. tamurae*, as validated species. *R. monacensis* has been so far reported from *Ixodes ricinus*, and *R. tamurae* from *Amblyomma testudinarium* [39]. The human pathogenic role of *R. monacensis* was first reported in Spain [18], and one case of *R. tamurae* infection has been detected in Japan [40]. Nevertheless, no evidence of human pathogenicity is presented herein for *Rickettsia* sp. 12G1, and there is no evidence to suggest that this *Rickettsia* is transmissible to humans. Other new genotypes with unknown pathogenicity that also belong to the same lineage of *R. tamurae* and *R. monacensis*, such as...

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**Table 5 Detection rates for *Rickettsia* spp. and *Anaplasma* spp.**

| Host/sampling | Tick species | Detection rate% (number of infected ticks/number of total ticks) |
|---------------|--------------|---------------------------------------------------------------|
|               |              | *Rickettsia* spp. | *A. phagocytophilum* | *A. marginale* | *Anaplasma* spp. |
| Andean tapir  | *A. multipunctum* | 0 | 1.4 (1/71) | 0 | 0 |
|               | *Amblyomma* spp. | 0 | 0 | 0 | 0 |
| Cattle        | *R. microplus* | 5.3 (3/57) | 1.4 (8/57) | 3.5 (2/57) | 5.3 (3/57) |
|               | *Ixodes* spp. | 0 | 0 | 0 | 0 |
|               | *I. boliviensis* | 0 | 0 | 0 | 0 |
| Vegetation    | *A. multipunctum* | 0 | 6.3 (1/16) | 0 | 0 |
| Total         |              | 2 (3/151) | 6.6 (10/151) | 1.3 (2/151) | 2 (3/151) |

*A. phagocytophilum*: *Anaplasma* phagocytophilum; *A. marginale*: *Anaplasma* marginale; *A. multipunctum*: *Amblyomma* multipunctum; *R. microplus*: *Rhipicephalus* microplus; *I. boliviensis*: *Ixodes* boliviensis.
Rickettsia sp. strain Colombianensi or Rickettsia sp. strain IbR/CRC, have been documented in R. microplus or I. boliviensis, from the New World [41,42].

In our study, A. phagocytophilum or closely related genotypes have been detected in ticks removed from Andean tapirs, cows and vegetation. It is known that the high intraspecific variability observed in the msp2 gene of A. phagocytophilum promotes the adaptation of the bacterium to different hosts and could justify its distribution in various environments [43]. As expected, the msp2 sequences obtained in this study (corresponding to 10 ticks) showed high genetic variability. Whereas the 16S rRNA sequences matched, when available (n = 2), with A. phagocytophilum pathogenic for humans (GenBank accession no. CP000235), msp2 sequences for 5 specimens (1 A. multipunctum from an Andean tapir and 4 R. microplus from cows) demonstrated relatedness with human pathogenic A. phagocytophilum but differed by 0.7-3.4% [44,45]. In addition, msp2 sequences obtained from 4 R. microplus were closest (96.6-98.3% identity) to A. phagocytophilum from Japanese Ixodes persulcatus [46]. Lastly, the msp2 sequence for 1 A. multipunctum from vegetation was 99% identical to one A. phagocytophilum strain from rodents in Florida (also highly similar to human pathogenic reference strain) [47].

As far as we know, the occurrence of A. phagocytophilum or closely related genotypes has not been previously detected neither in Ecuador nor in ticks removed from tapirs. Nevertheless, A. phagocytophilum or closely related Anaplasma spp. have been found in blood samples from domestic (dogs and cats) and wild animals (deer) in Brazil [48-50]. This is the first evidence of A. phagocytophilum in R. microplus in the New World. Nevertheless, this bacterium had been previously found in R. microplus from China [51].

Based on the sequencing results of the 16S rRNA gene, 2 R. microplus specimens removed from cows tested positive for A. marginale and 3 harbored Anaplasma spp. (assigned to A. marginale, A. ovis, A. phagocytophilum and A. centrale).

A. marginale, which is transmitted by R. microplus, has a worldwide occurrence and is considered as one of the most prevalent pathogens causing cattle morbidity and mortality in subtropical and tropical countries, including Latin America [52,53]. Our study evidences the first molecular detection of A. marginale in R. microplus from Ecuador. This bacterium had been previously detected in Ecuadorian blood samples from cattle by PCR [54] and also in R. microplus ticks in Philippines [55]. Moreover, no evidence of Ehrlichia spp. or B. burgdorferi s.l.-infected ticks had been found in Ecuador. Nevertheless, in South American countries, new members of the Ehrlichia genus and the B. burgdorferi s.l. complex have been recently described in Brazil, Uruguay and Chile [56-59].

Conclusions

In summary, this is the first description of Rickettsia sp. in ticks from Ecuador, and the analyses of sequences suggest the presence of a potential novel Rickettsia species. The complete characterization and distribution of the novel Rickettsia sp. 12G1, as well as its possible pathogenic role for animals and humans, needs to be determined.

Our data also showed that ticks from Andean tapirs, cattle and vegetation in Ecuador (Amblyomma and Rhipicephalus) were naturally infected with Anaplasmataceae and that co-infection (A. marginale and Rickettsia sp.) occurred.

Competing interests

The authors declare they have no competing interests.

Authors’ contributions

Designed the study: JAD, AP. Collected and identified ticks: CP, AMP. Processed samples and analysed msp2 sequences: CP, AMP. Analyzed the data: AP, AMP, JAD. Wrote the paper: AP, AMP, JAD. All authors read and approved the final version of the manuscript.

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References

1. Sonenshine DE, Lane RS, Nicholson WL. Ticks (Ixoida). In: Mullen G, Durden L, editors. Medical and Veterinary Entomology. New York: Academic Press; 2002. p. 517–58.
2. Guglielmone AA, Estrada-Peña A, Keitans JE, Robbins RG. Ticks (Acari: Ixodida) of the Neotropical Zoogeographic Region. Houten: Atalanta; 2003.
3. Barros-Battesti D, Arzua M, Bechara GH. Carrapatos de Importância Médico-Veterinária da Região Neotropical: Um Guia Ilustrado para Identificação de Espécies. Sao Pablo: Vox/ICTED-3/ Butantan; 2006.
4. Spolidorio MG, Andreoli GS, Martins TF, Brandão PE, Labruna MB. Rickettsial infection in ticks collected from road-killed wild animals in Rio de Janeiro, Brazil. J Med Entomol. 2012;49:1510–4.
5. André MR, Durnier JS, SCIPIO DG, Teixeira RH, Allegretti SM, Machado RZ. Molecular detection of tick-borne bacterial agents in Brazilian and exotic captive carnivores. Ticks Tick Borne Dis. 2012;3:247–53.
6. Barbosa da Silva J, Vinhote WM, Oliveira CM, André MR, Machado RZ, Da Fonseca AH, et al. Molecular and serological prevalence of Anaplasma marginale in water buffaloes in northern Brazil. Ticks Tick Borne Dis. 2014;5:100–4.
7. Díaz AG, Castellanos A, Piñeda C, Downer C, Lizcano DJ, Constantino E, et al. Tapires y II Congreso Ecuatoriano de Mastozoología, Puyo, Pastaza (Ecuador), May 8–11, 2013 (oral communication I-CLT 048), and in the IV Congreso Latinoamericano de Enfermedades Rickettsiales, San José (Costa Rica), July 22–24, 2013 (abstracts A-17 and B-13).
8. Labruna MB, Whitworth T, Bouyer DH, McBride J, Camargo LM, Camargo EP, et al. Rickettsia bellii and Rickettsia amblyommii in Amblyomma ticks from...
the State of Rondônia, Western Amazon, Brazil. J Med Entomol. 2004;41:1073–81.
9. Guglielmone AA, Beati L, Barros-Battesti DM, Labruna MB, Nava S, Venzl JM, et al. Ticks (Ixodidae) on humans in South America. Exp Appl Acarol. 2006;40:383–100.
10. Szabó NP, Pintér A, Labruna MB. Ecology, biology and distribution of spotted-fever tick vectors in Brazil. Front Cell Infect Microbiol. 2013;3:27.
11. Londoño AF, Díaz FJ, Valbuena G, Gazi M, Labruna MB, Hidalgo M, et al. Infection of Amblyomma ovale by Rickettsia sp. strain Atlantic rainforest, Colombia. Ticks Tick Borne Dis. 2014;5:672–5.
12. Fairchild GB, Kohls GM, Tipton VJ. The ticks of Panama (Acarina: Ixodidae). In: Wenzel WR, Tipton VJ, editors. Ectoparasites of Panama. Chicago: Field Museum of Natural History. 1966. p. 167–219.
13. Jones EK, Clifford CM, Keirans JE, Kohls GM. The ticks of Venezuela (Acarina: Ixodidae) with a key to the species of Amblyomma in the western hemisphere. Brigham Young University Science Bulletin, Biological Series. 1972;7:1–40.
14. Black WC, Piesman J. Phylogeny of hard- and soft-tick taxa (Acari: Ixodidae) based on mitochondrial 16S rDNA sequences. Proc Natl Acad Sci U S A. 1994;91:10034–8.
15. Beati L, Keirans JE. Analysis of the systematic relationships among ticks of the genera Rhipicephalus and Boophilus (Acari: Ixodidae) based on mitochondrial 12S ribosomal DNA gene sequences and morphological characters. J Parasitol. 2001;87:52–48.
16. Zähler M, Gothe R, Rinder H. Genetic evidence against a morphologically suggested conspecificity of Dermacentor reticulatus and Dermacentor marginatus (Acari: Ixodidae). Int J Parasitol. 1995;25:1413–9.
17. Weisburg WG, Barsm SM, Pelletier DA, Lane DJ. – 16S ribosomal DNA amplification for phylogenetic study. J Bacteriol. 1991;173:697–703.
18. Jado I, Oteo JA, Aldazmir M, Gil H, Escudero R, Ibarra V, et al. Rickettsia monacensis and human disease. Spain. Emerg Infect Dis. 2007;13:1405–7.
19. Labruna MB, Whitworth T, Horta MC, Bouyer DH, McBride JM, Pinter A, et al. Rickettsia species infecting Amblyomina cooperi ticks from an area in the state of São Paulo, Brazil, where Brazilian spotted fever is endemic. J Clin Microbiol. 2004;42:90–8.
20. Roux V, Fourrier PE, Raoult D. Differentiation of spotted fever group rickettsiae by sequencing and analysis of restriction fragment length polymorphism of PCR-amplified DNA of the gene encoding the protein rOmpA. J Clin Microbiol. 1996;34:2058–219.
21. Regnery RL, Spruill CL, Plikaytis BD. Genotypic identification of rickettsiae and human disease, Spain. Emerg Infect Dis. 2007;13:1405–7.
22. Choi YJ, Lee SH, Park KH, Koh YS, Lee KH, Baik HS, et al. Evaluation of a PCR-based assay for diagnosis of spotted fever group rickettsiosis in human serum samples. Clin Vaccine Immunol. 2005;12:759–63.
23. Sekeyova Z, Roux V, Raoult D. Phylogeny of Rickettsia sp. strain Colombianensi. J Clin Microbiol. 1998;36:1090–9.
24. Zähringer M, Spierer MA, Bonner JC, Reisen WK, Raoult D. – Global proteomic analysis of two tick-borne emerging zoonotic agents: Anaplasma phagocytophilum and Rickettsia helvetica. J Proteome Res. 2012;11:2422–35.
25. Watanabe Y, Nishijo K, Oda Y. – Relative abundance of Rickettsia spp. inferred by rOmpA. J Clin Microbiol. 1996;34:2058–219.
26. Zeidner NS, Burkot TR, Massung R, Nicholson WL, Dolan MC, Rutherford JS, et al. Detection of an undescribed Rickettsia sp. in ixodes boulengeri from roots of crops from the eastern United States. J Med Entomol. 2012;49:960–5.
27. Alvear V, Bonilla R, Chacón I. – Relative abundance of Rickettsia spp. in bovines (Bos taurus and B. indicus) from Costa Rica. Rev Biol Trop. 2003;51:415–27.
28. Paciora P, Paddock CD, Socolovkch V, Labruna MB, Medianikov O, Kornf T, et al. – Update on tick-borne rickettsioses around the world: a geographic approach. Clin Microbiol Rev. 2013;26:657–702.
29. Alvear V, Bonilla R, Chacón I. – Relative abundance of Rickettsia spp. in bovines (Bos taurus and B. indicus) from Costa Rica. Rev Biol Trop. 2003;51:415–27.
30. Miranda J, Portasio A, Oteo JA, Matar S. Rickettsia sp. strain Colombiansen (Rickettsiales: Rickettsiaceae). A New Proposed Rickettsia Detected in Amblyomma dissimilis (Acari: Ixodidae) from iguanas and free-living larvae ticks from vegetation. J Med Entomol. 2012;49:960–5.
31. Tzuev OV. A review of Neotropical Amblyomma species (Acari: Ixodidae). Acarina. 2007;153:1–134.
32. Labruna MB, Martin TF, Nunes PH, Costa FB, Portero F, Venzl JM. New records of Amblyomma multipunctum and Amblyomma respondens from Ecuador, with description of A. multipunctum nymph. J Parasitol. 2013;99:973–7.
33. Massung R, Slater K, Owens JH, Nicholson WL, Mather TN, Solberg VB, et al. Detection of Anaplasma phagocytophilum in humans and a novel human and veterinary pathogen. J Clin Microbiol. 2003;41:672–5.
34. Varon D, Velázquez IM, Chacón I. – Relative abundance of Rickettsia spp. in bovines (Bos taurus and B. indicus) from Costa Rica. Rev Biol Trop. 2003;51:415–27.
35. Raoult D, Fournier PE, Eremeeva M, Graves S, Kelly PJ, Oteo JA, et al. Naming of Rickettsiae and rickettsial diseases. Ann N Y Acad Sci. 2005;1063:1–12.
36. Labruna MB, Guglielmone AA. Ticks of New World Tapi. Tapir Conservation. 2009;18:218–21.
37. Zhao CL, Muro JJ, Clavijo JJ. Garrapatas del género Ixodes Lateville, 1795 y Rhipicephalus (Boophilus) Koch, 1844 (Acari: Ixodidae) presentes en la colección de Zoología Agrícola, Decanato de Agronomía, UCLa, Lara, Venezuela. Entomotropica. 2011;26:89–97.
38. Neumann LG. Révision de la famille des Ixodides. Mémoires de la Société de Zoologie de France. 1899;12:211–7.
39. Volzit OV. A review of Neotropical Amblyomma species (Acari: Ixodidae). Acarina. 2007;153:1–134.
54. Soto KK. Determinación de la prevalencia de anaplasmosis en el ganado bovino faenado en la empresa metropolitana de Rastro de Quito (EMRQ) mediante la aplicación de las técnicas de diagnóstico: microscopía de frotis sanguíneos, reacción en cadena de la polímerasa (PCR) y ensayo inmunoenzimático competitivo (cELISA). In: PhD thesis. Sangolquí: Escuela Politécnica del Ejército; 2010.

55. Ybañez AP, Sivakumar T, Ybañez RH, Ratilla JC, Perez ZO, Gabotero SR, et al. First molecular characterization of Anaplasma marginale in cattle and Rhipicephalus (Boophilus) microplus ticks in Cebu, Philippines. J Vet Med Sci. 2013;75:27–36.

56. Cabezas-Cruz A, Vancová M, Zweygarth E, Ribeiro MF, Grubhoffer L, Passos LM. Ultrastructure of Ehrlichia mineirensis, a new member of the Ehrlichia genus. Vet Microbiol. 2013;167:455–8.

57. Zweygarth E, Schöl H, Lis K, Cabezas-Cruz A, Thiel C, Silaghi C, et al. In vitro culture of a novel genotype of Ehrlichia sp. from Brazil. Transbound Emerg Dis. 2013;60:86–92.

58. Barbieri AM, Venzal JM, Marcelli A, Almeida AP, Gonzalez EM, Labruna MB. Borrelia burgdorferi sensu lato infecting ticks of the Ixodes ricinus complex in Uruguay: first report for the Southern Hemisphere. Vector Borne Zoonotic Dis. 2013;13:147–53.

59. Ivanova LB, Tomova A, González-Acuña D, Munsá R, Moreno CK, Hernández C, et al. Borrelia chilenis, a new member of the Borrelia burgdorferi sensu lato complex that extends the range of this genospecies in the Southern Hemisphere. Environ Microbiol. 2014;16:10669–80.