Role of Birds in Dispersal of Etiologic Agents of Tick-borne Zoonoses, Spain, 2009

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We amplified gene sequences from Anaplasma phagocytophilum, Borrelia garinii, B. valaisiana, B. turdi, Rickettsia monacensis, R. helvetica, R. sibirica sibirica, and Rickettsia spp. (including Candidatus Rickettsia vini) in ticks removed from birds in Spain. The findings support the role of passerine birds as possible dispersers of these tick-borne pathogens.

Hard ticks are a major vector of infectious diseases in industrialized countries. Several tick-borne bacterial diseases, such as Lyme disease, Mediterranean spotted fever, and tick-borne lymphadenopathy (also called Dermacentor-borne necrosis erythema and lymphadenopathy), are endemic to Spain. Furthermore, a few cases of human anaplasmosis and Rickettsia monacensis infection in humans have been diagnosed in Spain (1–3).

Birds are the preferred host for some tick species. As carriers of infected ticks, birds could be responsible for the spread of tick-borne bacteria that cause human anaplasmosis, Lyme disease, rickettsioses, and other diseases (4). Multiple studies support the conclusion or propose the hypothesis that birds play a role as reservoirs of Anaplasma phagocytophilum, Borrelia burgdorferi, and Rickettsia spp. (4–6). Because the Iberian Peninsula plays a major role in the migratory routes of birds, we aimed to determine the presence and prevalence of A. phagocytophilum, B. burgdorferi sensu lato, and Rickettsia spp. in ticks removed from birds captured in northern Spain.

The Study

During April–October 2009, bird bandings were conducted in the protected area of Finca Ribavellosa in La Rioja, Spain (42°14′N, 2°54′W). Ticks were collected from birds and classified through taxonomic keys (7) and molecular methods (8). DNA was individually extracted by using 2 incubations of 20 minutes each with ammonium hydroxide (1 mL of 25% ammonia and 19 mL of Milli-Q water that had been autoclaved) at 100°C and 90°C.

DNA extracts were used as templates for PCRs targeting fragment genes for tick classification and for bacteria detection (Table 1). Two negative controls, 1 containing water instead of template DNA and the other with template DNA but without primers, and a positive control (a tick extract, A. phagocytophilum, B. burgdorferi sensu stricto, or R. slovaca) were included in all PCRs. Amplification products were sequenced, and nucleotide sequences were compared with those available in GenBank by using a BLAST search (www.ncbi.nlm.nih.gov/blast/Blast.cgi). Phylogenetic and molecular evolutionary analyses were conducted by using MEGA4 (16 in online Technical Appendix, wwwnc.cdc.gov/EID/pdfs/11-1777-Techapp1.pdf).

A total of 222 ticks belonging to the species Haemaphysalis punctata (n = 1), Ixodes gregorii (n = 7), I. arboricola (n = 26), I. ricinus (n = 181), and other Ixodes spp. (n = 7) were collected from 97 passerine birds. Two nucleotide sequences for the 16S rRNA fragment gene of I. arboricola ticks were recorded (GenBank accession nos. JF791812 and JF791813) (Table 2).

A. phagocytophilum was detected only in 1 larva of an I. ricinus tick (0.5%). Twenty-nine (13.1%) samples tested positive for B. burgdorferi s.l. The most prevalent genospecies was B. garinii (n = 19), which was detected in I. ricinus (n = 16), H. punctata (n = 1), I. frontalis (n = 1), and Ixodes sp. (n = 1) ticks. B. valaisiana was amplified in 9 samples (8 I. ricinus and 1 Ixodes sp. ticks). B. turdi was found in 1 I. frontalis tick. Rickettsia infection was detected in 39 (17.6%) ticks. R. monacensis (n = 1), R. helvetica (n = 1), R. sibirica sibirica (n = 1), and Rickettsia spp. (n = 9) were detected in 12 I. ricinus ticks. Furthermore, according to gltA, ompA, and ompB sequence analysis, a possible new Rickettsia sp. was found in 25 I. arboricola ticks and 2 I. ricinus ticks. For these 27 samples, highest identities with R. heilongjiangensis (97.1%) and R. japonica (99.1%) were found for ompA (GenBank accession no. JF758828) and ompB (GenBank accession no. JF758826) nucleotide sequences, respectively, whereas gltA nucleotide sequences were identical to those from both Rickettsia spp. According to multilocus sequence typing (data not shown) and genetic criteria agreed on by experts, a Candidatus status could be assigned. We named it Candidatus Rickettsia vini (17 in online Technical Appendix) (Table 2). The phylogenetic
Table 1. PCR primer pairs used in study of the role of birds in dispersal of etiologic agents of tick-borne zoonoses, Spain, 2009*

| Bacteria              | Gene target         | Primer name | Primer sequence, 5’ → 3’ | Amplified fragment, bp | Annealing temp., °C | Ref. |
|-----------------------|---------------------|-------------|---------------------------|------------------------|---------------------|------|
| **Anaplasma spp.**    | 16S rRNA, nested    | ge3a        | CACATGCAAGTCTGAGGATATTCC  | 932                    | 55                  | (9)  |
|                       |                     | ge10r       | TTTCCGTAAGAAGGATCTAATCT   | 546                    | 55                  | (9)  |
|                       |                     | ge9f        | AACCGATTTTCTTTGACCTG     | 546                    | 55                  | (9)  |
|                       | msp                 | ge2         | GGCAGTATCTGAAAGCGTCCAGG  | 334                    | 56                  | (10) |
|                       |                     | msp3F       | CCAGGCGTTTAGGAAATGAGAAG  | 334                    | 56                  | (10) |
|                       |                     | msp3R       | GCCGAGTAAACCATCATAAGG    | 334                    | 56                  | (10) |
| **Borrelia spp.**     | flaB, nested†       | Outer 1     | AARGAATTGGCAGTTCAATC     | 497                    | 52                  | (11) |
|                       |                     | Inner 1     | ACATATTCAGTGACAGAGTTCTTA| 389                    | 55                  | (11) |
|                       |                     | 5S-23S is   | GAAAGGTCTGAGCGCCGCTG     | 380                    | 52                  | (12) |
|                       |                     | 23SC1       | TAAGCTGACTAATACTACCC     | 380                    | 52                  | (12) |
|                       |                     | 23SN1       | ACCATAAGCTTTATTTTACG     | 226                    | 55                  | (12) |
|                       |                     | 23SN2       | ACCATAGACTCTTATATTGC     | 226                    | 55                  | (12) |
| **Rickettsia spp.**   | **ompA** seminested | Rr190.70p   | ATGGCGAATATTTCTCCAAAA    | 631                    | 46                  | (13,14) |
|                       |                     | Rr190.701n  | GTTCCGGAATATTTCTCCAAAA  | 532                    | 48                  | (14) |
|                       |                     | Rr190.602n  | AGTGCAGCATTCGCTCCCC     | 532                    | 48                  | (14) |
|                       |                     | rompB OF    | GTAACCGGAAGTCGTTTCGTAA  | 511                    | 54                  | (15) |
|                       |                     | rompB OR    | GCTTTAATACGCTAACCC      | 420                    | 56                  | (15) |
|                       |                     | rompB SFG/TG| GGTTTGCCCATTACCCATTAG   | 381                    | 48                  | (14) |
|                       |                     | gltA central| GGCGGCTGCTACGCGGCC      | 381                    | 48                  | (14) |
|                       |                     | region, nested| GAGTGAGGCGGTTGCGACG     | 337                    | 54                  | (15) |
|                       |                     | I. arboricola| GGCAGGTATACCCATTAGCA    | 337                    | 54                  | (15) |
|                       |                     | I. arboricola| GGCGGCTGCTACGCGGCC      | 381                    | 48                  | (14) |
|                       |                     | I. arboricola| GAGTGAGGCGGTTGCGACG     | 337                    | 54                  | (15) |
|                       |                     | I. arboricola| GGCGGCTGCTACGCGGCC      | 381                    | 48                  | (14) |

*Temp., temperature; ref., reference; msp, p44 major surface protein gene; flaB, flagellin gene; rompB, 120-kDa genus common antigen gene; ompA, 190-kDa protein antigen gene; gltA, citrate synthase gene.
†R = A/G; W = A/T.

Table 2. Anaplasma phagocytophilum, Borrelia burgdorferi s.l., and Rickettsia spp. detected in ticks removed from birds, Spain, 2009*

| Tick Bacteria        | Tick Species | Tick Stage | Bird (no. specimens) | Gene targets |
|----------------------|--------------|------------|----------------------|--------------|
| **A. phagocytophilum** | Ixodes ricinus | 1 L        | T. merula (1)         | msp          |
| **B. garinii**       | I. ricinus   | 4 L, 2 N   | T. merula (9)         | flaB         |
|                      |              | 3 L, 4 N   |                      | flaB         |
|                      |              | 1 L        | E. rubecula (1)       | flaB         |
|                      |              | 1 L        | T. philomelos (1)     | flaB         |
|                      |              | 1 L        | Trogodytes troglodytes (1) | flaB |
|                      |              | 1 L        | T. philomelos (1)     | flaB         |
|                      |              | 1 L        | E. rubecula (1)       | flaB         |
|                      |              | 1 L        | T. merula (1)         | flaB         |
|                      |              | 2 L        | T. philomelos (2)     | flaB         |
|                      |              | 1 L        | E. rubecula (1)       | flaB         |
|                      |              | 1 L        | Garrulus glandarius (1) | flaB |
| **B. valaisiana**    | Ixodes spp.  | 1 L        | T. merula (1)         | flaB         |
|                      | I. ricinus   | 1 L, 1 N   | T. merula (3)         | flaB         |
|                      |              | 2 L        |                      | flaB         |
|                      |              | 1 L, 1 N   | T. philomelos (2)     | flaB         |
|                      |              | 1 L        | E. rubecula (1)       | flaB         |
|                      |              | 1 L        | Garrulus glandarius (1) | flaB |
| **B. turdi**         | I. frontalis | 1 L        | T. merula (1)         | flaB         |
| **R. monacensis**    | I. ricinus   | 1 L        | S. atricapilla (1)    | ompA         |
| **R. helvetica**     | I. ricinus   | 1 L        | G. glandarius (1)     | gltA         |
| **R. sibirica sibirica** | I. ricinus | 1 L        | S. atricapilla (1)    | ompA         |
| **Rickettsia spp.†** | I. ricinus   | 1 L, 1 L   | T. philomelos (1)     | ompA or gltA|
|                      |              | 4 L        | E. rubecula (4)       | ompA or gltA|
|                      |              | 2 N        | T. merula (2)         | gltA         |
|                      |              | 1 L        | Tr. troglodytes (1)   | gltA         |
| **Candidatus Rickettsia vini** | I. arboricola | 20 N | Cyanistes caeruleus (1) | ompA, ompB, gltA |
|                      |              | 5 L        | Parus major (1)       | ompA, ompB, gltA |
|                      |              | 2 L        | E. rubecula (2)       | ompA, ompB, gltA |

*L, larva; msp, p44 major surface protein gene; N, nymph; flaB, flagellin gene; 5S-23S is, 5S-23S rRNA intergenic spacer; rompB, 120-kDa genus common antigen gene; ompA, 190-kDa protein antigen gene; gltA, citrate synthase gene.
†Same identity with >1 validly published Rickettsia species.
tree based on ompA gene shows the nearest relationships among Rickettsia spp. (Figure).

Two I. ricinus larvae showed co-infection with B. garinii and Rickettsia sp. One nymph was co-infected with B. valaisiana and Rickettsia sp.

Conclusions

The presence of Anaplasma, Borrelia, and Rickettsia species in ticks removed from passerine birds corroborates the role of these vertebrates in the epidemiology and dispersion of tick-borne pathogens in Spain and in other zones of the planet. Some of the parasitized birds in our study, such as the European robin (Erithacus rubecula) or Eurasian blackcap (Sylvia atricapilla), are considered migratory or partial migratory birds. In addition, these species share an ecologic niche and ectoparasites (horizontal transmission) with other migratory birds that cover long distances from Africa to the Eurasian region.

Except for I. arboricola, the tick species captured in this study previously had been found on birds in Spain (18 in online Technical Appendix). Nevertheless, I. arboricola ticks are commonly hosted by birds. The high prevalence of I. ricinus ticks was expected because it is the most frequent tick in this area, and the immature stages of this tick frequently parasitize birds.

I. ricinus ticks are the main vectors of A. phagocytophilum in Europe, and this microorganism has been detected on vegetation in the studied area (1). However, the low prevalence (0.5%) of A. phagocytophilum in the ticks in our study corroborates data from other studies (19,20 in online Technical Appendix). The presence of A. phagocytophilum in a larva in our study supports the role of birds as reservoirs of A. phagocytophilum.

The prevalence (13.1%) of B. burgdorferi in our samples is similar to prevalences reported in other studies in Europe in which I. ricinus is the main species of tick captured from birds (19 in online Technical Appendix). In Spain, B. garinii, B. valaisiana, and B. afzelii have been detected in ticks from birds (18 in online Technical Appendix). According to our data, the human pathogen B. garinii was the most prevalent species, as reported in birds from Europe (21 in online Technical Appendix). B. turdi was discovered in Asia. Although it has been recently detected in ticks from birds in Norway (22 in online Technical Appendix), its finding in Spain was unexpected.

Regarding Rickettsia species, R. monacensis and R. helvetica are among the human pathogens detected in our study. Both species have been identified in ticks from birds in Europe (19,20,23 in online Technical Appendix). On the contrary, Candidatus Rickettsia vini, a potential new Rickettsia species, also detected in our study, has not been related to human disease (17 in online Technical Appendix). Several genospecies closely related to R. heilongiangensis and R. japonica have been identified in Ixodes spp. ticks removed from birds (23 in online Technical Appendix). R. sibirica sibirica, responsible for Siberian tick typhus in western People’s Republic of China and in Siberia, was also amplified in an I. ricinus larva in this study.

Our data confirm the involvement of birds in the cycle of human tick-borne diseases. The findings confirm that birds can disperse vectors and microorganisms.

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Technical Appendix

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