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Draft *Anaplasma phagocytophilum* Genome Sequences from Five Cows, Two Horses, and One Roe Deer Collected in Europe

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***Anaplasma phagocytophilum* is a zoonotic tick-borne intracellular bacterium responsible for granulocytic anaplasmosis. As it is difficult to isolate and cultivate, only 20 *A. phagocytophilum* genomes have been sequenced to date. Here, we present eight *A. phagocytophilum* genome sequences obtained using alternative approaches based on sequence capture technology.**

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Anaplasma phagocytophilum is a zoonotic and obligate intracellular bacterium transmitted by hard ticks. It is the causative agent of tick-borne fever in ruminants, a disease which causes significant economic losses in Europe, and of equine granulocytic anaplasmosis (EGA) in horses, of canine granulocytic anaplasmosis (CGA) in dogs, and of human granulocytic anaplasmosis (HGA) in both the United States and Europe (1). *A. phagocytophilum* is difficult to isolate and cultivate, and thus only 20 genomes have been sequenced to date, among which only four originate from European samples. In particular, there are no *A. phagocytophilum* genomes from horse or roe deer samples, even though EGA can have such important economic impact (2), and roe deer are suspected to play a crucial role as reservoir hosts in *A. phagocytophilum* epidemiology (1). Here, we present the draft genome sequences of European *A. phagocytophilum* from four cow and two horse samples, and two strains isolated from one cow and one roe deer which had been maintained in continuous cell cultures (3, 4).

To overcome limitations due to the lack of reliable, easy, and

feasible protocols to isolate *A. phagocytophilum*, we decided to follow a different strategy in order to obtain these genome sequences. We used a whole-genome sequence capture approach, whose efficacy on *A. phagocytophilum* has already been demonstrated (5). For each sample, total genomic DNA was extracted from whole blood of the animal, or from bacterial culture in IDE8 tick cell lines, using the NucleoSpin Blood QuickPure kit (Macherey-Nagel, Bethlehem, USA). The six bacterial genomes from animal blood were then captured and sequenced as previously described (5), but in this case a liquid-phase protocol based on the SeqCap EZ library kit (NimbleGen, Madison, USA) was used instead of solid-phase. All eight samples were sequenced on a single flow cell lane of a HiSeq3000 (Illumina, San Diego, USA) sequencer as paired-end 150 bp reads (insert-size: 280 ± 50 bp).

First, overlapping paired-end reads were merged with Flash (6) and were trimmed with Sickle (<https://github.com/najoshi/sickle>) (−n −q 24 −l 100). Reads were digitally normalized using Khmer (7) with a k-mer size of 20 and a cutoff of 20. Then, the

TABLE 1 Genome sequence accession numbers

| Sample | Nucleotide sequence accession no. | Host | Geographical origin | Genome size (bp) | No. of contigs (scaffolds) | G+C (%) | No. of genes |
|------------|-----------------------------------|----------|---------------------|------------------|----------------------------|---------|--------------|
| Cow_1 | FLLR01000001–FLLR01000249 | Cow | France | 1,682,137 | 249 | 41.93 | 1,519 |
| Cow_2 | FLMA01000001–FLMA01000230 | Cow | France | 1,641,350 | 230 | 42.23 | 1,463 |
| Cow_3 | FLMB01000001–FLMB01000191 | Cow | France | 1,561,654 | 191 | 42.00 | 1,417 |
| Cow_4 | FLLZ01000001–FLLZ01000230 | Cow | France | 1,604,419 | 230 | 42.12 | 1,448 |
| Cow_5 | FLMD02000001–FLMD02000300 | Cow | Germany | 2,196,284 | 300 | 43.70 | 1,979 |
| Horse_1 | FLMF02000001–FLMF02000300 | Horse | France | 1,784,294 | 300 | 41.90 | 1,657 |
| Horse_2 | FLMC02000001–FLMC02000300 | Horse | France | 2,191,611 | 300 | 41.58 | 1,997 |
| Roe_deer_1 | FLME02000001–FLME02000300 | Roe deer | Germany | 2,120,290 | 300 | 42.72 | 1,912 |

remaining reads were mapped to the host genomes [*Bos taurus* (UMD3.1), *Equus caballus* (EquCab2.0), and *Capreolus capreolus* (CCMK000000000)] and to *A. phagocytophilum* HZ (NC_007797.1), using the BWA algorithm (v0.6.1) with default parameters (8). Only those reads mapping to *A. phagocytophilum* or which remained unmapped were retained. Three sets of reads were used for assembly processing: paired-end reads mapped on *A. phagocytophilum*, paired-end unmapped reads, and singleton reads (unmapped or mapped to *A. phagocytophilum*). Those reads were then assembled using Spades (9), with a k-mer value from 81 to 125 with a step of 4, and default parameters. Genome annotation was performed using Prokka. Genome characteristics are summarized in Table 1.

Accession number(s). The eight draft genomes have been deposited in the European Nucleotide Archive (Table 1).

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