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# Molecular Detection of a New *Anaplasma* Species Closely Related to *Anaplasma phagocytophilum* in Canine Blood from South Africa

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**Canine DNA samples from South Africa were found to contain 16S rRNA gene nucleotide and citrate synthase gene nucleotide and deduced amino acid sequences that were most similar to *Anaplasma phagocytophilum*: 98%, 66%, and 69% similarity, respectively. This suggests that a new *Anaplasma* species closely related to *A. phagocytophilum* occurs in Africa.**

Ehrlichioses are tick-borne diseases that cause significant morbidity and mortality in dogs and people worldwide. In South Africa, canine ehrlichiosis is encountered commonly in veterinary practice (15, 17) and there is evidence of human ehrlichiosis (13). Currently, there are eight species in the family *Anaplasmataceae* that are pathogens in dogs: *Ehrlichia canis*, *Ehrlichia chaffeensis*, *Ehrlichia ewingii*, *Ehrlichia ruminantium*, *Anaplasma platys*, *Anaplasma phagocytophilum*, *Neorickettsia helminthoeca*, and *Neorickettsia risticii*. Although canine ehrlichiosis was first recognized in South Africa in 1938 (9), the organisms causing infections in the country have yet to be isolated and characterized. Serological studies, however, have shown that many dogs (up to 75%) have significant antibody titers against *E. canis* and *E. chaffeensis* (12). Also, DNA of *E. canis* and DNA of a novel *Ehrlichia* sp. closely related to *E. ruminantium* have been found in the blood of dogs in South Africa (1). There is also little definitive data on infections in dogs in other parts of Africa. The initial description of *E. canis* was made in dogs in Algeria in 1935 (5), and DNA of *A. platys* has been found in blood from a dog in the Democratic Republic of Congo (16).

Unfortunately, detecting members of the family *Anaplasmataceae* is not easy as they are fastidious intracellular bacteria, and their isolation and characterization require sophisticated laboratory facilities and substantive funding which are not generally available in Africa. While serological studies can be performed in less equipped laboratories, their results are hindered by antigenic cross-reactivity between the etiological agents. Molecular techniques, however, have been shown to be sensitive and specific in detecting infections with members of the family *Anaplasmataceae* (10, 14), and in this report we describe our use of PCR and sequence analysis to investigate the presence of these organisms in dogs in South Africa.

In 2000, morulae were observed in stained (Diff-QuicK; Harleco) central blood smears from three dogs (SA1108, SA1076, and SA1245) which presented at the Veterinary Teaching Hospital of the Medical University of South Africa. A QIAamp tissue kit (QIAGEN GmbH, Hilden) was used to extract DNA from whole blood collected in EDTA from each dog. The DNA samples were stored at −20°C in 200 μl of Tris-EDTA buffer until screened with a PCR using primers EHR16SD and EHR16SR, which amplify a 345-bp fragment of the 16S rRNA gene found in the genera *Anaplasma*, *Ehrlichia*, *Neorickettsia*, and *Wolbachia* of the family *Anaplasmataceae* (11). The three samples revealed appropriate amplicons, and for further phylogenetic studies, we carried out PCRs with primer sets fD1/EHR16SR and EHR16SD/Rp2, which amplify a longer sequence of the 16S rRNA gene (11), and with primers ANA-CS646F (5′ TGCATGCAGATCATGAAC 3′) and ANA-CS1076R [5′ GAGTAAAA(A or G)TCAACATT(G or C or T)GG 3′], which amplify a 431-bp partial sequence of the *Anaplasma* citrate synthase gene (*gltA*). Direct sequencing of the PCR products and analysis of the sequences obtained were performed as described previously (8). The GenBank accession numbers of the 16S rRNA gene sequences used to construct phylogenetic trees and to analyze percent identities were as follows: *A. phagocytophilum*, M73220, M73223, M73224, U02521, U10873, AF036645 to -036647, AF093788, AF093789, AF172164 to -172167, AF189153, AF241532, AJ242784, AY527213, and AY527214; *A. platys*, M82801, AF286699, AF287153, AF303467, AF536828, and AY530806; *A. bovis*, U03775, AF294789, AF470698, and AY144729; *A. ovis*, AF309865, AF318945, and AF414870; *Anaplasma* sp. found in a sheep in South Africa (Omatjenne), U54806; *Anaplasma* sp. found in a goat in Mozambique (Bom Pastor), AF318023; *A. marginale*, AF414871; *A. centrale*, AF414869; *E. canis*, M73221; *Ehrlichia ruminantium*, AF325175; *Wolbachia pipientis*, AF179630; and *N. risticii*, M21290. The GenBank accession

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TABLE 1. Percent similarities between the 16S rRNA gene sequences detected in dogs SA1108, SA1076, and SA1245 in South Africa and those reported for *Anaplasma* and other related species

| Species or dog (GenBank accession numbers) | % 16S rRNA gene similarity | |
|---|---|---|
| | SA1108 | SA1076 and SA1245 |
| SA1108 | 100 | 99.93 |
| SA1076 and SA1245 | 99.93 | 100 |
| *A. phagocytophilum* Webster (U02521) | 98.56 | 98.49 |
| *A. platys* (AF303467) | 98.13 | 98.06 |
| *Anaplasma* sp. from goat in Mozambique (AF318023) | 97.84 | 97.77 |
| *Anaplasma* sp. from sheep in South Africa (U54806) | 97.77 | 97.69 |
| *A. bovis* (U03775) | 96.83 | 96.90 |
| *A. marginale* (AF414871) | 96.18 | 96.11 |
| *A. ovis* (AF414870) | 95.89 | 95.82 |
| *A. centrale* (AF414869) | 95.97 | 95.89 |
| *E. canis* (M73221) | 91.85 | 91.77 |
| *W. pipientis* (AF179630) | 88.11 | 88.04 |
| *N. risticii* (M21290) | 85.37 | 85.30 |

numbers of the *gltA* nucleotide sequences used in our study were as follows: *A. phagocytophilum*, AF304136 to -304138, and AY464132 to -464138; *A. platys*, AB058782, AF478130, AY077620, and AY530807; *A. marginale*, AF304140; *A. centrale*, AF304141; *E. canis*, AF304143; *E. ruminantium*, AF304146; *W. pipientis*, AF332584; and *N. risticii*, AF304147.

The 1,389-bp sequences of the 16S rRNA gene we obtained with the DNA extracted from the blood of the three dogs were identical (for dogs SA1076 and SA1245) or very similar (99.30%) (Table 1). They had the highest levels of similarity to sequences reported for other *Anaplasma* spp. and lower levels of similarity to *E. canis*, *W. pipientis*, and *N. risticii* (Table 1). Among the *Anaplasma* spp., the greatest levels of similarity were with strains of *A. phagocytophilum*, but these were always less than 99%. The percentages of similarity between the sequences derived from the South African dogs and *Anaplasma* from southern Africa (Omatjenne from a sheep [2] and Bom Pastor from a goat [4]) were also relatively low, ranging from 97.69 to 97.84%. In the phylogenetic tree inferred from the 16S rRNA gene data, the sequences obtained from the South Africa dogs grouped together to form a distinct cluster which was most closely related to sequences reported for *A. phagocytophilum*, *A. platys*, and *Anaplasma*, identified in ruminants in South Africa, and *A. bovis* (Fig. 1). The bootstrap values we determined, however, were low and were only poorly supportive of these relationships. The above data indicates that the dogs we studied were infected with an *Anaplasma* species that is different from other reported *Anaplasma* spp. but most closely related to *A. phagocytophilum*.

Analyses of similarity and phylogenetic relationships using partial nucleotide and deduced amino acid sequences of the *gltA* were also performed for a representative of the organisms identified in the South African dogs (SA1108) (Table 2). Although bootstrap values were again low, in the phylogenetic trees based on the *gltA* data, the *Anaplasma* sp. in the South African dog was distinct from *A. phagocytophilum* and *A. platys* (Fig. 2). The percent similarities between SA1108 and *A. phagocytophilum* for the *gltA* nucleotide and deduced amino

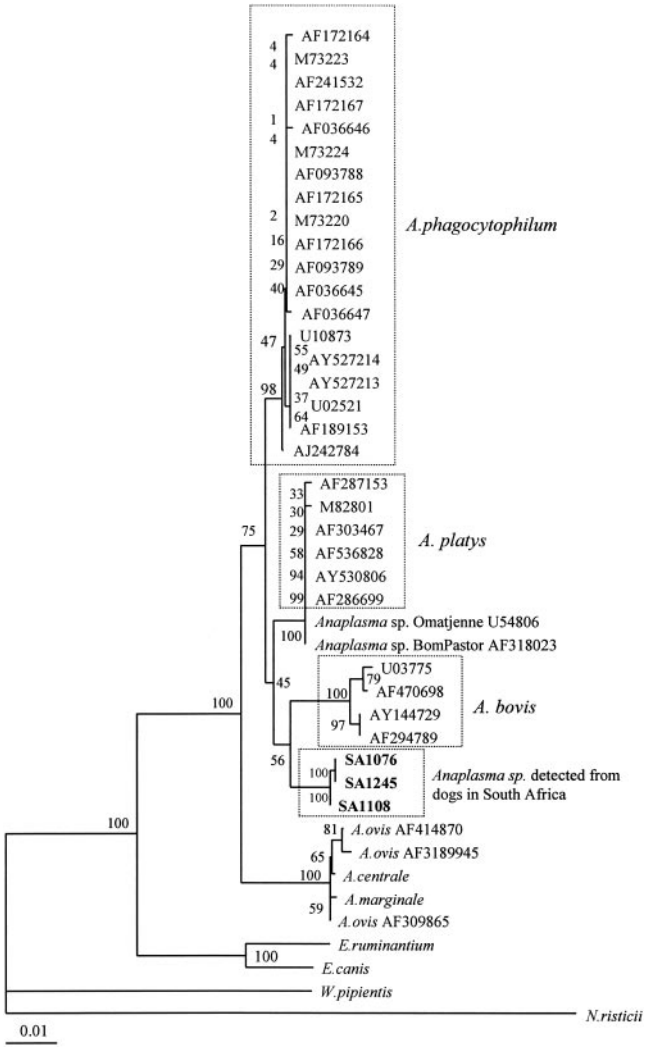


FIG. 1. Phylogenetic relationships between the new *Anaplasma* sp. detected in this study and *Anaplasma*, *Ehrlichia*, *Wolbachia*, and *Neorickettsia* based on partial nucleotide sequences of the 16S rRNA gene. The numbers at nodes are the proportions of 100 bootstrap resamplings that support the topology shown. The scale bar represents 10% divergence.

TABLE 2. Percent similarity of nucleotide and deduced amino acid sequence of the citrate synthase gene detected in dog SA1108 and those reported in *Anaplasma* and other species

| Species (GenBank accession no.) | % Identity to SA1108 citrate synthase gene | |
|---|---|---|
| | Nucleotide | Amino acid |
| *A. phagocytophilum* Webster (AF304136, AAL01656) | 66.44 | 69.47 |
| *A. centrale* (AF304141, AAL01659) | 63.67 | 68.42 |
| *A. marginale* (AF304140, AAL01658) | 63.32 | 66.32 |
| *A. platys* (AB058782, AAB87841) | 62.98 | 65.26 |
| *E. canis* (AF304143, AAL01661) | 62.98 | 66.32 |
| *E. ruminantium* (AF304146, AAL01664) | 64.01 | 63.16 |
| *W. pipientis* (AF332584, AAL57051) | 59.52 | 60.00 |
| *N. risticii* (AF304147, AAL01665) | 57.80 | 55.91 |

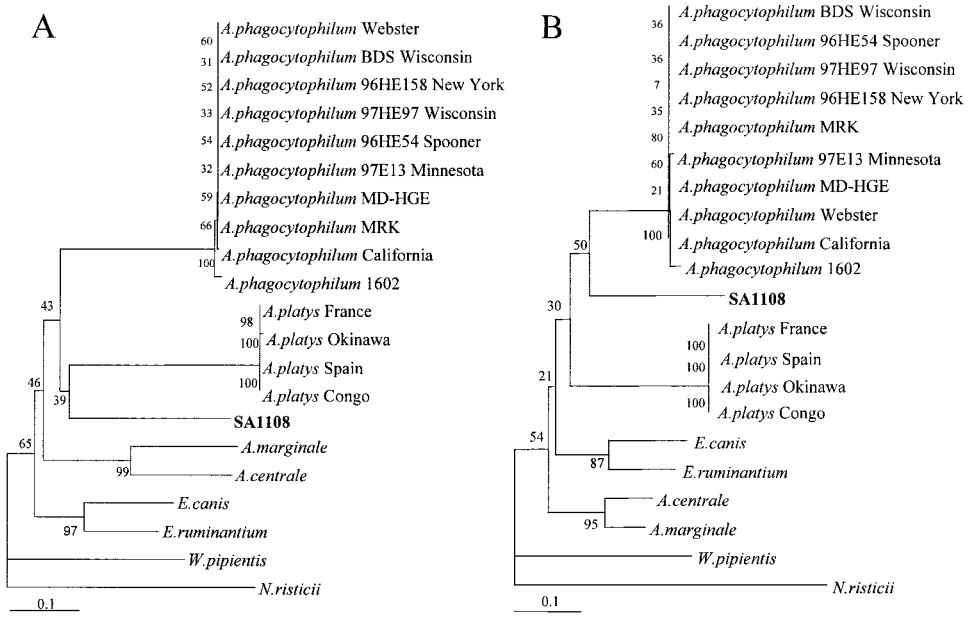


FIG. 2. Phylogenetic relationships between the new *Anaplasma* sp. detected in this study and *Anaplasma*, *Ehrlichia*, *Wolbachia*, and *Neorickettsia* based on partial nucleotide sequences of *gltA* (A) and deduced amino acid sequences of *gltA* (B). The numbers at nodes are the proportions of 100 bootstrap resamplings that support the topology shown. The scale bar represents 10% divergence.

acid sequences were only 66.44 and 69.47%, respectively (Table 2). These results are consistent with our findings using analyses of the 16S rRNA gene sequences.

Our report is the first describing an *Anaplasma* sp. occurring in dogs in South Africa. The new *Anaplasma* sp. we detected is most closely related to *A. phagocytophilum*, which is an agent of canine (6, 7) and human (3) granulocytic anaplasmosis in the United States and Europe. Also, it is closely related to *A. platys*, which may cause cyclic thrombocytopenia in dogs and is known to occur in Africa (10). Organisms closely related to *A. platys* and to the new *Anaplasma* sp. have been detected in the blood of a goat in Mozambique that died with clinical signs suggesting heartwater due to *E. ruminantium* (4). They have also been found in a sheep in South Africa which also had signs of heartwater and was concurrently infected with *E. ruminantium* (2). Our finding of an *Anaplasma* sp. in dogs in South Africa adds to the scant information on the *Anaplasmataceae* that occur in Africa. Unfortunately, records containing the laboratory and clinical data on the dogs in which we identified the novel *Anaplasma* sp. have been lost. Additional studies are indicated to further characterize the organism and to determine its pathogenicity in dogs and perhaps other species, its distribution, vectors, and epidemiology.

**Nucleotide sequence accession number.** The 16S rRNA gene sequences obtained from dogs SA1076, SA1108, and SA1245 have been deposited in the GenBank database under accession numbers AY570539, AY570538, and AY570540, respectively. The *gltA* sequence obtained from dog SA1108 has been registered under accession number AY570541.

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