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Relationship of Molecular and Clinical Findings on *Anaplasma phagocytophilum* Involved in Natural Infections of Dogs▽

Clinical manifestations of canine granulocytic anaplasmosis (CGA), caused by *Anaplasma phagocytophilum*—an obligate intracellular tick-transmitted bacterium infecting neutrophilic granulocytes—range from self-limiting to severe. Commonly, lethargy, inappetence, and fever and, further, lameness, pale mucous membranes, tense abdomen, diarrhea, vomiting, surface bleeding, tachypnea, enlarged lymph nodes, and splenomegaly are observed. Laboratory abnormalities may include thrombocytopenia, anemia, lymphopenia, neutrophilia, neutropenia, leukocytosis, leukopenia, monocytosis, lymphocytosis, hypoalbuminemia, hyperglobulinemia, increased liver enzymes, and hyperbilirubinemia. Subclinical infections without clinical signs are also possible (1, 2). *A. phagocytophilum* also causes disease in horses, cattle, sheep, and humans and is found in a large number of potential wild reservoir hosts (7). It shows genetic heterogeneity, e.g., when isolated from different host species or geographic origins (4, 5). No significant differences were observed in previous studies concerning the rates of seropositivity or PCR positivity in dogs with and without clinical signs of CGA (3). We hypothesized that different 16S rRNA gene variants of *A. phagocytophilum* may be involved in natural infection of dogs, as has been shown in natural infection of horses (6) and that those might cause different clinical outcomes. Thus, DNA was extracted with the Qiagen DNA Minikit (Qiagen, Hilden, Germany) according to the manufacturer's instruction from 66 frozen *A. phagocytophilum*-positive blood samples from dogs originating in Germany ($n = 64$), Sweden ($n = 1$), and Switzerland ($n = 1$), which were diagnosed as positive for *A. phagocytophilum* in routine diagnostic procedures or epidemiological screenings from 2003 to 2010. The full clinical data were available, and coinfection with other vector-borne infections was excluded by PCR (data not shown). A nested PCR targeting the partial 16S rRNA gene (497 bp), followed by sequencing, was performed as previously described (5). Most frequently obtained was variant B ($n = 37$; the amplified part equals the prototype sequence of the human granulocytic anaplasmosis agent [GenBank accession no. U02521]), followed by variant A ($n = 23$; equals Frankonia 2 [accession no. AF136712]). In 6 dogs, 5 other variants were detected (one dog was infected with two variants simultaneously), but those were not included in the comparative analysis (FJ829788, FJ829789, JN656381, JN656383, FJ829790, FJ829791). Selected clinical or laboratory abnormalities were investigated concerning their relationship to sequence variants A and B using Fisher's exact test (GraphPad Prism 5). Statistically significant differences ($P < 0.05$) were observed for fever, lethargy, inappetence, thrombocytopenia, and lymphopenia, which all appeared more frequently with variant B than with variant A, even though both variants caused clinical disease (Table 1). These clinical and laboratory abnormalities often appear together in CGA. Open questions remain. Is variant A less pathogenic for dogs, or did the acute phase of the disease pass unnoticed? Were the subclinical dogs in this study persistently infected? It is not yet clear whether the results indicate that partial 16S rRNA gene variants A and B are expressions of different strains of *A. phagocytophilum* with different pathogenicities for dogs. Further characterization of the variants is thus warranted, and variants should be studied further in *in vitro* and *in vivo* experiments.

TABLE 1. Comparative analysis of selected clinical and laboratory parameters and *A. phagocytophilum* 16S rRNA gene variants A and B occurring in natural infections of 60 dogs

Investigated parameter and occurrence	No. of cases with sequence variant:		Significant difference (P value)
	A[h]	B[i]	
Fever			
Yes	5	26	Yes (<0.0001)
No	16	11	
Lethargy			
Yes	10	30	Yes (0.0037)
No	12	6	
Inappetence			
Yes	8	25	Yes (0.0166)
No	14	11	
Lameness			
Yes	6	8	No
No	16	30	
Splenomegaly			
Yes	7	17	No
No	3	11	
Thrombocytopenia[a]			
Yes	11	28	Yes (0.0226)
No	11	7	
Leukopenia[b]			
Yes	0	6	No
No	22	28	
Lymphopenia[c]			
Yes	2	18	Yes (0.0265)
No	8	10	
Anemia[d]			
Yes	7	18	No
No	15	17	
Monocytosis[e]			
Yes	6	14	No
No	4	16	
Increased ALP value[f]			
Yes	5	21	No
No	10	10	
Hypoalbuminemia[g]			
Yes	4	13	No
No	9	17	

[a] Defined as a platelet count of <180,000/μl.
[b] Defined as a white blood cell count of <6,000/μl.
[c] Defined as a lymphocyte count of <1,000/μl.
[d] Defined as a hematocrit of <0.40 mmol/liter.
[e] Defined as a monocyte count of >500/μl.
[f] Defined as an alkaline phosphatase concentration of >97 IU/liter.
[g] Defined as an albumin concentration of <28 g/liter.
[h] GenBank accession no. FJ829748 to FJ829750, FJ829752, FJ829753, FJ829755, FJ829756, FJ829758 to FJ829762, JN656336 to JN656340, and JN656342 to JN656346.
[i] GenBank accession no. FJ829764 to FJ829766, FJ829768, FJ829770, FJ829772 to FJ829774, FJ829777 to FJ829779, FJ829781 to FJ829784, FJ829786, JN656352 to JN656371, JN656378, and JN656380.

We have no conflict of interest to declare.

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