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IS GRANULOCYTIC EHRLICHIOSIS A ZOONOSIS, TRANSMITTED BY *IXODES RICINUS* TICKS?

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Ehrlichia DNA dans des échantillons de sang d' animaux suédois et dans des tiques Ixodes ricinus a été analysé par séquençage du gène de 16S rRNA. Trois différentes séquences d'Ehrlichia ont été identifiées dans les tiques, une d'entre elles étant identique à l'agent de l' ehrlichiose humaine granulocytaire (HGE). Toutes les séquences dans les échantillons de chien et de cheval étaient identiques à celle de l'agent du HGE. Les échantillons bovins contenaient deux séquences différentes, une étant identique à E. phagocytophila. Les divergences en séquences étaient très petites et toutes localisées près de la fin 5´de la molécule du 16S rRNA. Ces résultats indiquent que les tiques peuvent être vecteurs d'un Ehrlichia sp. (avec la séquence de l' agent du HGE), qui cause une maladie chez des animaux et, selon des donnés sérologiques, aussi chez l'Homme.

BACKGROUND

Ehrlichiae, with a tropism for granulocytes, are known to cause tick-borne fever in cattle and sheep and granulocytic ehrlichiosis in horses and dogs (EGE and CGE, respectively). Recently, a similar disease was also described in humans (HGE) in the US. The ehrlichiae responsible for these infections, *E. phagocytophila*, *E. equi* and the HGE agent, are closely related and belong to the same genogroup. Nucleotide sequence analysis of the 16S rRNA gene of Swedish *Ehrlichia* isolates from clinical cases of EGE and CGE showed that the sequence was identical to that of the American HGE agent. In a seroepidemiological study of humans in Sweden, antibodies to the HGE agent has been demonstrated, but no clinical case of HGE has been confirmed. In Europe, *Ixodes ricinus* ticks are known to transmit tick-borne fever and it is suspected that *Ixodes* ticks are also vectors for EGE and CGE. Since the HGE agent has been demonstrated in *Ixodes* ticks in the US it was considered important to study Swedish ticks in order to assess their potential as vectors for presumably zoonotic granulocytic ehrlichiosis.

MATERIALS AND METHODS

Blood samples from animals were collected from different parts of southern and central Sweden, including the coastal areas. *Ixodes ricinus* ticks were collected from the Stockholm area and Koster Islands on the west coast. DNA was extracted by a phenol-chloroform procedure (blood samples) or by use of QIAamp Tissue Kit (QIAGEN, Hilden, Germany) (ticks). DNA amplification was performed with primers complementary to 16S rRNA gene sequences of the *E. phagocytophila* genogroup. Nucleotide sequencing of PCR products were either performed by cycle sequencing in ABI PRISM 373 Sequencer (Perkin- Elmer Applied Biosystem, CA, USA) (tick samples), or by solid- phase DNA sequencing with the A.L.F. DNA Sequencer (Pharmacia Biotech, Uppsala, Sweden) (blood samples).

RESULTS

In samples from dogs and horses (n=16), so far only one sequence type has been found and this one is identical to that of the HGE agent. In bovines, either *E. phagocytophila* sequence or a sequence differing in two nucleotide positions have been obtained. PCR products from 14 ticks have been sequenced. One nymph contained ehrlichial DNA with the HGE agent sequence. In four ticks (3 nymphs, 1 adult) a sequence differing in only one nucleotide position from that of the HGE agent, and in 9 ticks (6 nymphs, 3 adults) a sequence differing in one position from that of *E. phagocytophila* were obtained. In all, five different ehrlichia sequences with only small sequence divergencies have been demonstrated in samples from Swedish ticks and animals. The nucleotide differences were all located in the variable V1 region close to the 5´end of the 16S rRNA molecule. These results confirm the ability of Swedish ticks to harbour ehrlichiae belonging to the *E. phagocytophila* genogroup.

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