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Detection of the agent of human granulocytic ehrlichiosis (HGE) in UK ticks using polymerase chain reaction

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SUMMARY

Nymphal *Ixodes ricinus* ticks collected from woodland areas in South Wales, UK, were tested using the polymerase chain reaction for the presence both of the causative agent of human granulocytic ehrlichiosis (HGE) and *Borrelia burgdorferi*. Twenty-two of 60 (37%) ticks were found positive in the PCR for *B. burgdorferi* and 4/60 (7%) for the HGE agent. One tick was found positive both for *B. burgdorferi* and HGE agent. Our findings imply the presence of the HGE agent in UK ticks and the finding of a tick apparently containing both pathogens underlines the potential for concurrent infection with HGE agent and *B. burgdorferi* to occur after a single tick-bite. Based on our observations, we conclude that there may be a need to consider a range of pathogens both in laboratory investigation and clinical management of suspected tick-borne disease in the UK, particularly where there is a clinical presentation atypical of Lyme borreliosis alone.

INTRODUCTION

Human granulocytic ehrlichiosis (HGE) was first described in 1994 in the mid-western US [1]. The agent of HGE, although not yet fully characterized, appears to be genetically related most closely to the species *Ehrlichia equi* and *Ehrlichia phagocytophila* [2], both well recognized as causes of veterinary disease. Symptoms of HGE include fever, granulocytopaenia, thrombocytopaenia, and some cases have resulted in death. The HGE agent appears to be transmitted to man by Ixodid ticks and it has been suggested that it may have similar ecological requirements to *Borrelia burgdorferi* s.l., the causative agent of Lyme disease.

The presence of the HGE agent in European ticks has recently been confirmed [3], and an ehrlichia with an identical 16S ribosomal RNA gene sequence has also been identified in dogs and horses in Sweden [4]. Indirect evidence for the presence of HGE in the human population of Europe has been suggested by serological studies in Norway [5], Sweden [6], Switzerland [7] and the UK [8]. The serological test employed in the UK study was based on immunofluorescence microscopy using *E. phagocytophila* as the antigen for detection of antibodies to the HGE agent. The presence of the closely related *E. equi* in the UK has also been suggested after a report of equine granulocytic ehrlichiosis in a horse in Somerset [9]. A key aim of the present study was to determine whether the presence of HGE agent could be confirmed in UK ticks by direct detection using the polymerase chain reaction.

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MATERIALS AND METHODS

Tick collection and processing for PCR

Ticks collected by blanket dragging from a forested location in South Wales, UK were tested for the presence of both HGE agent and *Borrelia burgdorferi* using polymerase chain reaction. Individual nymphal ticks were each placed in a 0·2 ml micro-tube

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(Advanced Biotechnologies Ltd Surrey, UK) and macerated in 100 μl NH_4OH (0·5 mol/l). Each sample was incubated at 100 °C for 5 min with the tube closed and 10 min with the tube open. 10 μl of each sample was then added to a 50 μl PCR assay for subsequent DNA amplification.

PCR for detection of *B. burgdorferi*

Two pairs of 'nested' PCR primers were constructed complementary to the gene encoding the OSP-A protein of *B. burgdorferi* strain B13 [10]. Primer pair 1, OspA-N1 (5′-GAGCTTAAAGGAACTTCTGATAA-3′) OspA-C1 (5′-GTATTGTTGTACTGTAATTGT-3′), and primer pair 2, OspA-N2 (5′-ATGGATCTGGAGTACTTGAA-3′) OspA-C2 (5′-CTTAAAGTAACAGTTCCTTCT-3′), correspond to nucleotide nos. 334–356, 874–894, 362–381 and 693–713, respectively, of the OspA gene. The primary PCR reaction mix contained 1·25 mmol l^{-1} $MgCl_2$, and 1 μmol l^{-1} each of primers OspA-N1 and OspA-C1. PCR amplification was carried out for 50 cycles using a denaturing temperature of 94 °C, annealing temperature of 37 °C and extension temperature of 72 °C. 0·5 μl of each sample was transferred to a second PCR reaction mix in which the starting primers were replaced with primers OspA-N2 and OspA-C2 and amplified for 30 cycles. Detection of any PCR products was by ethidium bromide staining after agarose gel electrophoresis.

PCR for detection of HGE agent

Two pairs of 'nested' primers used previously for the detection of HGE agent in Ixodid ticks were constructed [11]. Gene amplification was carried out as described above for *B. burgdorferi* except that the *E. phagocytophila*/*E. equi*-specific primer pairs Ehr521 (5′-TGTAGGCGGTTCGGTAAGTTAAAG-3′), Ehr747 (5′-GCACTCATCGTTTACAGCGTG-3′) and Ehr552 (5′-GCCAGGGCTTAACCCTGGAGC-3′), Ehr706 (5′-TCCTGTTTGCTCCCCACGCTTTC-3′) were substituted for the *B. burgdorferi*-specific primer pairs OspA-N1, OspA-C1, and OspA-N2, OspA-C2 respectively.

RESULTS AND DISCUSSION

Sixty nymphal ticks were tested for the presence of both HGE agent and *B. burgdorferi* s.l. using the polymerase chain reaction. Twenty-two (37%) ticks were found to be positive for *B. burgdorferi* and 4 (7%) for HGE agent. One tick (1·7%) was found to be co-infected with *B. burgdorferi* and HGE agent.

The prevalence of infection by *B. burgdorferi* in ticks collected in South Wales is comparable to that reported previously in nymphal ticks throughout southern England [12]. The detection by PCR of the HGE agent in Ixodid ticks confirms the risk of infection both to the rural population and visitors to woodland areas of South Wales. The finding of a tick containing both *B. burgdorferi* and HGE agent underlines the potential for co-infection in humans resulting from a single tick-bite. The clinical significance and potential sequelae of such co-infections is presently unclear. The direct detection of HGE agent in ticks in the present study, and the finding of co-infection with both HGE agent and *B. burgdorferi* underlines the need to consider a range of pathogens both in laboratory investigation and clinical management of suspected tick-borne disease in the UK, particularly where the clinical presentation is atypical of Lyme borreliosis alone [13].

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