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Field evidence that roe deer (*Capreolus capreolus*) are a natural host for *Ehrlichia phagocytophila*

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SUMMARY

Samples of blood, spleen and legs from 112 culled roe deer (*Capreolus capreolus*) were collected from nine sites widespread in the United Kingdom. The prevalence of infection with *Ehrlichia phagocytophila* was determined by serology and polymerase chain reaction. Means of 58% of 102 plasma or serum samples were seroreactive by IFA, 38% of 84 blood samples and 29% of 82 spleen samples were positive by PCR. Ticks on legs of 71 roe deer were *Ixodes ricinus* larvae, nymphs and adults and 83% of legs were infested. Numbers of ticks corresponded positively to the percentage of samples positive for *E. phagocytophila* by serology and PCR for different sampling sites. *Ixodes ricinus* nymphs collected from the vegetation at one site with infected deer were analysed for infection with *E. phagocytophila* by examination of Feulgen stained salivary glands. Of 135 nymphs 5% were infected. These results confirm that roe deer are commonly parasitized by both *E. phagocytophila* and its vector tick in such a way that it is likely to be an important natural mammalian reservoir of *E. phagocytophila*.

INTRODUCTION

Ehrlichia phagocytophila is an obligate intracellular Gram-negative rickettsia causing tick-borne fever (TBF), which is transmitted by *Ixodes ricinus* ticks [1, 2]. It infects neutrophils producing non-specific symptoms such as fever, abortion and immunosuppression and is endemic in those sheep and cattle populations of the United Kingdom that graze pasture infested with *I. ricinus*. Little is known about the prevalence of exposure and the effects of this pathogen in wild ruminants but there is evidence of infection of deer by *Ehrlichia* species [3]. Results from inoculation of blood from roe and fallow deer (*Dama dama*) from the New Forest in England, into susceptible cattle and sheep [4], and from red deer from the island of Rhum in Scotland into susceptible sheep [2] indicate that these deer in the United Kingdom can be infected with *E. phagocytophila* to produce classical TBF in cattle and sheep. No quantitative data was provided by these authors but it was assessed that fallow and roe deer are 'probably the prime hosts for this micro-organism rather than the sheep' [4]. White-tailed deer are a reservoir for *E. chaffeensis* in the United States [5], and this species also harbours granulocytic *Ehrlichia* sp. [6, 7]. *Ehrlichia phagocytophila* is genetically and serologically closely related to the agent of human granulocytic ehrlichiosis (HGE) [8]. The HGE agent is an emerging pathogen that infects humans, horses and dogs in North America and continental Europe [9–11].

The distribution of deer as an important maintenance host for tick reproduction [12] is strongly associated with other tick-borne pathogens such as *Borrelia burgdorferi*, causative agent of Lyme borreliosis. With most *Borrelia* species rodents infested with larvae and nymphs of *Ixodes* ticks act as the main mammalian reservoir of infection [13]. However, sheep can maintain enzootic cycles of Lyme

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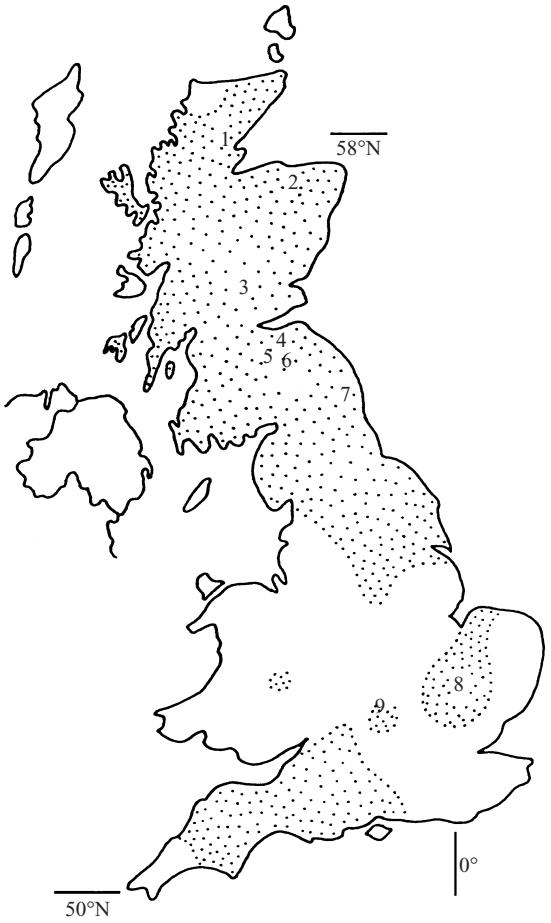

Fig. 1. The United Kingdom showing approximate location of sites where roe deer were sampled indicated by their numbers as given in Tables 1, 2, 3 and 5. Also shown is the approximate distribution of roe deer in the United Kingdom, redrawn from [18] as stippled areas.

disease spirochaetes in the absence of alternative hosts [14]. There is some evidence of the role of rodents in the transmission of *E. phagocytophila* in North America [15] and the United Kingdom [16]. Neither white-tailed deer (*Odocoileus virginianus*) in the United States [17] nor roe deer (*Capreolus capreolus*) in Europe [18] seem to be competent reservoirs for the Lyme borreliosis spirochaetes. Roe deer are susceptible to infection with louping-ill virus, but despite the deer seroconverting and showing histopathological changes their titres of patent viraemia do not approach the threshold required to infect ticks with the virus [19]. It is not known if roe deer are serving just as hosts for tick maintenance or whether they also contribute to ehrlichial infection of the tick population as competent reservoirs.

Roe deer are common and widely distributed in many areas of Scotland and England, but are absent or rare in Northern Ireland and Wales [20] (Fig. 1). Their preferred habitat is deciduous and coniferous woodland, and the latter habitat is widely distributed adjacent to upland sheep at risk from TBF. The use by humans of these habitats for recreation, forestry and agriculture is increasing and thus exposure to pathogens from deer may rise.

The aim of this study was to contribute to a better understanding of the epidemiology of ehrlichiosis in roe deer by quantifying its prevalence by serologic testing and PCR, and by providing field evidence for likely transmission between deer by ticks.

METHODS

Deer sampling

From November 1997 to November 1998 samples from roe deer of various ages and both sexes were obtained at regular intervals during legally regulated culls for woodland management at nine sites in the United Kingdom (Fig. 1). Serum samples from southern Scotland were also obtained during 1994–6 but whole blood or spleen for PCR was not available from these deer. Blood was collected from the iliac vein [21] as soon as possible after death, during gralloching the deer in the field. Blood for PCR was collected in 5 ml EDTA tubes and for IFAT in 10 ml heparinized tubes. A 5 g piece of spleen and the distal portion of foreleg (severed at the carpal joint) were collected from each deer and placed in separate plastic containers. Samples from each deer were transported to the laboratory in separate plastic containers to avoid cross-contamination.

Tick counts from deer legs

Immediately after arrival deer legs were stored at −20 °C. Ticks were collected by washing out the plastic bag and sieving the wash at 125 μm pore diameter. To collect ticks that remained attached, each leg was thawed and the hair separated with forceps in five transects along its length, aided by a stereoscopic microscope. All ticks found were identified and counted.

Tick collections from vegetation and examination for infection

To obtain an estimate of the prevalence of *E. phagocytophila* infection in the population of host-seeking ticks, in September 1998 nymphs and adults were collected from vegetation by dragging with a 1 m square cloth along grassy rides from two conifer

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Table 1. *Numbers of roe deer samples collected from nine widespread sites in the United Kingdom, separated according to sex and age*

	Sex		Age	
Site no.	Male	Female	Adult	Calf
1	1	0	1	0
2	10	8	13	5
3	3	5	7	1
4	1	1	2	0
5	15	19	20	14
6	2	1	2	1
7*	14	6	13	4
8	10	3	10	3
9	1	0	1	0
Total	57/100	43/100	69/97	28/97

* Discrepancies in the number of animals are due to lack of details from the sample.

forests (sites 5 and 7) on two occasions at each site, during similar mild and dry weather. Drags were 10 m long, took 25 s and were 10 m apart. Forty drags were made at site 5 and 45 at site 7. Salivary glands of nymphal and adult ticks were examined for infection with *E. phagocytophila* [22].

Indirect fluorescent antibody test

Plasma was harvested from heparinized blood after centrifugation at 1000 g for 15 min. Samples were tested for antibodies to *E. phagocytophila* by the indirect fluorescent antibody test (IFAT) [23]. Samples were screened at a dilution of 1/100 in PBS, pH 8·0, on spot slides of neutrophils infected with *E. phagocytophila* (feral goat isolate). Fluorescein isothiocyanate anti-sheep IgG (whole molecule) conjugate developed in donkey (Sigma) was diluted to 1/80 in PBS. Samples were considered positive when their titres were equal or higher to 100. Positive samples were two-fold diluted, serologic results were recorded as the reciprocal of the highest dilution at which specific fluorescence of ehrlichial morulae could be detected within infected neutrophils. Negative controls were from sheep with no history of TBF and positive controls were from the same sheep after experimental infection with *E. phagocytophila* using blood stabilates.

Immunoblotting

Solubilized proteins of *E. equi* grown in tick cell culture [24] were separated by sodium dodecyl sulphate polyacrylamide gel electrophoresis, SDS–PAGE [25]. Western blotting followed using nitrocellulose membranes (Hybond) which were incubated with 1/50 dilutions of 14 plasma samples from roe deer that were positive by IFAT at low and high titre. Positive and negative controls were included as for IFAT. Donkey anti-sheep IgG peroxidase conjugate (Sigma) was used at 1/500 dilution.

Molecular diagnosis

GroE operon gene sequences for *E. phagocytophila* and closely related bacteria were retrieved from Genbank™ computer database. Specific oligonucleotide primers for *E. phagocytophila* were designed using Lasergene DNAstar software program to amplify 410 bp PCR product of the *groE* gene. Primers HSP534 (TGTACTCAATAAGCTCCGTGGTG) and HSP1326 (CTACTCTGTCTTTGCGTTCCTTCA) were synthesized by Cruachem (Glasgow). Paired blood (anticoagulated with EDTA) and spleen samples were screened by PCR after DNA extraction using the QIAGEN tissue kit (QIAamp) following manufacturer's instructions. DNA was eluted with distilled water to a final volume of 200 μl for spleen and 100 μl for blood samples. For each reaction the PCR mixture contained 0·5 mM $MgCl_2$, 0·2 mM of each dNTP (Boehringer–Mannheim), 0·2 μM of each primer, 1·25 units of Taq DNA polymerase (Thermometric Ltd), plus 5 μl of 10x Ultrotaq buffer (Thermometric Ltd) and 5 μl of DNA template in a final volume of 50 μl onto which 50 μl of sterile mineral oil (Sigma) was layered. PCR amplification was performed using an Omnigene thermal cycler (Hybaid) with an initial denaturation step at 94 °C for 4 min followed by 35 cycles of denaturation at 94 °C for 45 s, annealing at 60 °C for 45 s, and extension at 72 °C for 45 s, followed by a final extension at 72 °C for 7 min.

As positive control, DNA was extracted (QIAgen tissue kit, QIAamp) from sheep blood, experimentally infected with *E. phagocytophila*, when characteristic morulae were observed in 32% of neutrophils by examination of Giemsa-stained blood smears. The detection threshold of the primers was determined by ten-fold serial dilutions of DNA in sterile distilled water to detect the minimum number of infected neutrophils for a positive signal.

To discard false-positive results, Southern blotting of spleen and blood samples from which a PCR product was amplified followed. A 410 bp fragment

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Table 2. *Numbers of different instars of* Ixodes ricinus *ticks* (*larvae, nymphs and adults*) *observed on roe deer legs from nine widespread sites in the United Kingdom. Mean numbers of ticks and range for each site are shown*

		Mean numbers of ticks per leg (range)		
Site	No. legs	Larvae	Nymphs	Adults (male)
1	1	29	6	0
2	14	11 (0–57)	4 (0–18)	0·07 (0–1)
3	10	26 (2–48)	14 (3–40)	0·1 (0–1)
4	2	2 (0–4)	0	0
5	8	0·8 (0–5)	0	0
6	2	21 (1–48)	3 (0–8)	0
7	15	63 (4–454)	25 (4–99)	0·07 (0–1)
8	18	74 (0–636)	10 (2–34)	0
9	1	0	0	0

derived from a sheep experimentally inoculated with *Ehrlichia phagocytophila* (feral goat isolate) was labelled using Boehringer–Mannheim labelling and detection kit following manufacturer's instructions. PCR positive samples from blood and spleen were electrophoresed in 1% agarose gels at 70 V in TBE buffer (89 mM Tris-borate, 2·5 mM EDTA) for 30 min. Five μl of a 1 kb ladder (Gibco) were included in the gel to confirm the PCR product size. The gels were recovered and assembled into a sandwich for blotting to positively charged Nylon membranes Hybond™ - N^+ (Amersham International) using Transblot® SD semi-dry elecrophoretic cell (Biorad Ltd). DNA was transferred to the membranes at a constant current of 3·55 mA/cm² for 10 min. Following transfer, the membranes were recovered and the DNA fixed with 0·2 M NaOH, then the membrane was baked at 80 °C for 30 min. Hybridization followed as described in the Hybridization Protocol booklet supplied by Boehringer–Mannheim.

Statistical analysis

Data were analysed using Epi-Info microcomputer software, v. 6.0 [26]. Exact binomial 95% confidence intervals (CI), kappa coefficient and χ^2 values were computed.

RESULTS

Deer samples

A total of 112 roe deer samples were examined from nine locations widespread across the United Kingdom (Fig. 1). Sex and age composition of the population of deer is shown in Table 1.

Ticks from deer

Of the 71 roe deer legs examined 59 (83%) were infested with ticks. All adult and nymphal ticks were *I. ricinus*. Larval ticks are difficult to identify but none characteristic of *I. trianguliceps, I. canisuga* or *I. hexagonus* were found (except for one *I. trianguliceps* from site 9). Thus it is assumed that the majority were *I. ricinus*. All instars of *I. ricinus* simultaneously parasitized roe deer, particularly during May to June. Nymphs were found from March through to November. There was wide variation in infestation levels between the sites, as shown in Table 2.

Ticks from vegetation and their infection

Ticks from vegetation at site 7 comprised 135 nymphs and 38 adults of *I. ricinus* (equivalent to 300 nymphs and 84 adults per 100 m² sampled). None of the adults but 7 nymphs (5%) had infections of *E. phagocytophila*. Only one *I. ricinus* was found at site 5, a female (equivalent to 2·5 adults per 100 m² sampled).

Indirect fluorescent antibody test

A total of 102 plasma/serum samples were tested by IFA. Of these, 59 had antibody titres reactive for *E. phagocytophila* ranging from 100 to 12800, giving an overall prevalence of 58% (Table 3). No significant differences in the prevalence of infection were found by age or sex of the deer, thus separate data tabulated by age or sex are not presented.

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Table 3. *Summary of IFAT serology for antibodies to* E. phagocytophila *in* 102 *roe deer samples collected from nine locations across the United Kingdom*

	IFAT			
Site	Point prevalence % (No. positive/no. sampled)	95% CI†	GM titre‡	Maximum titre
1	0 (0/1)	0–98	—	—
2*	83 (15/18)	59–96	2918	12800
3*	100 (8/8)	63–100	1745	3200
4	0 (0/2)	0–84	—	—
5*	26 (10/38)	13–43	230	6400
6	100 (3/3)	29–100	1600	3200
7	61 (11/18)	36–83	1096	3200
8*	92 (12/13)	64–99	2851	12800
9	0 (0/1)	0–98	—	—
All sites	58 (59/102)		1423	12800

* The difference in seroprevalence between the four sites was statistically significant, $\chi^2_{\text{d.f.}=3} = 32{\cdot}57$; $P < 0{\cdot}001$.
† Exact binomial 95% confidence intervals.
‡ Geometric mean. Data for samples negative at 1/100 dilution were omitted.

Table 4. *Roe deer samples* (*either blood or spleen*) *that appeared positive by PCR showing a fragment of the expected size* (*410 bp*) *from the groE operon, were compared to the number of animals that exhibited reactivity by IFAT to* E. phagocytophila. *The difference was statistically significant and kappa coefficient indicated only fair agreement between tests*

	PCR (blood or spleen)		
IFAT	+	−	
+	33	22	55
−	8	19	27
Total	41	41	82

Yates corrected $\chi^2_{\text{d.f.}=1} = 5{\cdot}52$; $P < 0{\cdot}05$; kappa coefficient, 0·27; observed agreement, 0·63; chance expected agreement, 0·5; standard error of kappa, 0·1; Z, 2·58; one-tailed P-value, 0·0049.

A high variation in prevalence of infection between sites was observed. The seroprevalence in sites 2, 3 and 8 was significantly higher than at site 5 ($\chi^2 = 32{\cdot}57$, $P < 0{\cdot}001$) (Table 3). Exact binomial 95% confidence intervals for the point prevalence in site 5 did not overlap with the confidence intervals of sites 2, 3 and 8 suggesting a true difference in seroprevalence. The small sample size from the sites 1, 4, and 6 did not allow making further comparisons. The high seroprevalence corresponded positively with higher percentages of PCR positive for *E. phagocytophila* in blood and spleen samples, as shown in Tables 3, 4 and 5. Comparison of results using the kappa statistic indicated fair agreement between PCR and IFAT (Table 4) (kappa = 0·27; $P < 0{\cdot}01$) [27]. As for individual animals the seropositivity did not always correlate to PCR-positive samples either in blood or spleen, although 80% of the PCR-positive animals were also positive by IFA and 60% of the seropositive animals were also positive by PCR (Table 4). Yates corrected χ^2 test for this table was significant ($\chi^2 = 5{\cdot}52$; $P < 0{\cdot}05$). Our sample included varied ages of deer that probably had different stages of ehrlichial infection, with varying antibody levels. The numbers of deer in groups of age and sex were insufficient to test this further.

Immunoblotting

Western blot analysis of roe deer samples that were reactive by IFAT confirmed their exposure to *E. phagocytophila* or a closely related *Ehrlichia* sp. (Fig. 2). The samples, included the positive control, reacted to a 44 kDa band specific for granulocytic ehrlichia [8, 28, 29].

Molecular diagnosis

PCR specific primers amplified a 410 bp product (Fig. 3) from *E. phagocytophila groE* gene but they did not amplify DNA from the closely related species *Cowdria ruminantium*, *Ehrlichia bovis* and *E. canis*. The primers were able to detect 10^{-3} dilution of DNA in sterile distilled water extracted from experimentally infected

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Table 5. *The results of testing for the presence of* E. phagocytophila groE *gene in roe deer blood and spleen samples by PCR and Southern blotting are compared. Some of the PCR products that appeared smaller than the controls were not recognized by a specific probe after Southern blotting and were considered negative*

	PCR		Southern blot			
	% (no. +)		% (no. +)		No. samples	
Site	Blood	Spleen	Blood	Spleen	Blood	Spleen
1	0 (0)	0 (0)	0 (0)	0 (0)	1	1
2	78 (14)	56 (10)	78 (14)	50 (9)	18	18
3	63 (5)	63 (5)	63 (5)	38 (3)	8	8
4	0 (0)	0 (0)	0 (0)	0 (0)	2	2
5	0 (0)	6 (1)	0 (0)	6 (1)	18	16
6	0 (0)	33 (1)	0 (0)	33 (1)	3	3
7	45 (9)	25 (5)	35 (7)	25 (5)	20	20
8	46 (6)	23 (3)	46 (6)	23 (3)	13	13
9	0 (0)	0 (0)	0 (0)	0 (0)	1	1
Total	41 (34)	33 (27)	39 (32)	30 (24)	84	82

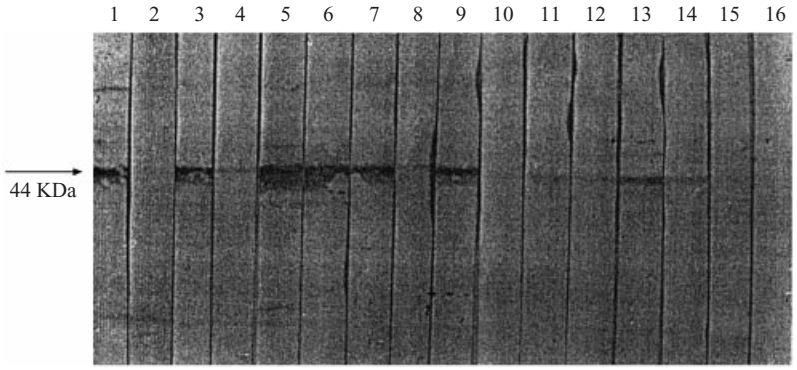

Fig. 2. Western immunoblot of roe deer sera that by IFAT, showed high and low but positive antibody titres to *E. phagocytophila* using *Ehrlichia equi* proteins as antigens. Molecular mass of the protein is expressed in kDa based on the low range prestained SDS–PAGE standards (Bio-Rad). Lane 1, serum from sheep experimentally inoculated with *E. phagocytophila*, 3 weeks after exposure. Lane 2, serum from the same sheep prior to inoculation. Lanes 3–9, roe deer samples with high titre to *E. phagocytophila*; Lanes 10–16, roe deer samples with low titre to *E. phagocytophila*. Positive samples reacted to a 44 kDa band (arrow), specific for granulocytic ehrlichia.

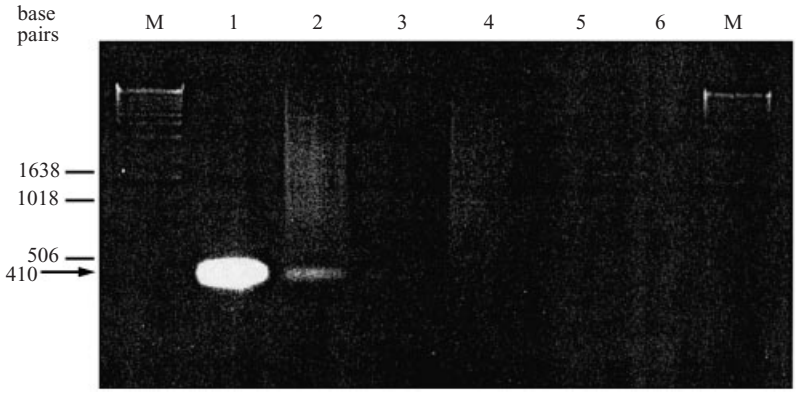

Fig. 3. Agarose gel electrophoresis of products amplified from roe deer blood and spleen by PCR with primers HSP534 and HSP1326. M, molecular size marker; Lane 1, *E. phagocytophila* DNA derived from experimentally infected sheep; lanes 2 and 4, samples of roe deer spleen; lanes 3 and 5, samples of roe deer blood; Lane 6, sterile distilled water Lanes 1, 2 and 3 are positive showing a fragment of the *groE* operon gene with the expected size of 410 bp (arrow).

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sheep blood, when 32% of the neutrophils were infected, which is beyond the level of visible detectable parasitaemia by microscopy.

Two PCR products derived from blood and three derived from spleen appeared to be a few base pairs smaller than the controls. Southern blotting was performed to confirm the specificity of the PCR product (Table 5). These samples were not recognized by the probe and were considered negative. Overall, 32 (38%) of blood and 24 (29%) of spleen samples were positive by PCR and confirmed by Southern blotting. Positive samples were amplified from roe deer culled from March to November.

DISCUSSION

In the United States between 8 and 15% white-tailed deer were found positive to granulocytic ehrlichia by serology and PCR respectively [7]. 16S rDNA sequence analysis showed that the ehrlichia was nearly identical to the published sequences for the agent of HGE, *E. phagocytophila* and *E. equi*. Our study found varied prevalences of infection in roe deer depending on the location of the sampling site, with an overall prevalence of 58% by IFAT and 38–29% by PCR in blood and spleen respectively. Our results corroborate initial observations based on blood inoculations [4]. They suggest a high prevalence of exposure and infection in roe deer to an agent that we also characterize as *E. phagocytophila* on the basis of the degree of specificity of PCR primers and comparison with positive control material from classical *E. phagocytophila* infections in sheep tested by Southern blotting. In addition, deer were infested with all instars of ticks, some of which were infected with an agent proven to be *E. phagocytophila* in a parallel study [22].

In this study the distribution of *E. phagocytophila* in roe deer populations appeared to be very patchy and corresponded positively with sites having high densities of *I. ricinus*. The high prevalence of deer blood samples positive by PCR suggest that the deer were recently exposed to the pathogen since it is not detectable by PCR in experimentally infected sheep after day 17 in whole blood or day 14 in serum post-inoculation (Alberdi, unpublished data). However, antibodies to tick-borne fever persist for several weeks in sheep, less long in cattle [30]. That may explain the high percentage of seropositive animals in areas with high tick densities representing either primary exposure or challenge. Positive animals by IFAT and PCR were also found at site 5 suggesting that tick-borne fever can be maintained in roe deer populations supporting very low tick densities. Site 7 showed a higher percentage of infected *I. ricinus* nymphs, 5%, than the 1–2% in our previous study [22]. Others have found variations in the same measure of 1·4–6% [16]. This suggests spatial variation in the prevalence of infection in ticks although it appears generally low.

Legs have been found effective for sampling ticks on deer [31], and we corroborate this as a convenient method. Observations by those handling the deer in this study suggested that *I. ricinus* on a leg represent a small proportion of the total on the whole body. Thus our evidence points to heavy infestations through much of the year on most roe deer.

Little is known about the pathogenesis of ehrlichiosis in wild ruminants. Although it is assumed to be mild, severe reactions to *E. phagocytophila* have been recorded during experimental infection of reindeer [32]. Roe deer suffer frequently disease from flukes (*Fasciola hepatica*) and lungworm (*Dictyocaulus* spp.) [33]. The immunosuppressive effects of infection with *E. phagocytophila* could possibly aggravate these or other infections. Blood infections of the roe deer detected by PCR coincided with tick infestations of the animals throughout March to November. This would promote onward transmission from deer to ticks. The capacity of *E. phagocytophila* to proliferate in roe deer and to infect *Ixodes* ticks feeding on the deer would be difficult to prove by experimental transmissions because roe deer are not farmed in the United Kingdom, in contrast to red deer. However, our circumstantial evidence for the involvement of roe deer in a natural endemic cycle of *E. phagocytophila* transmission follows. (i) High seroprevalence; (ii) high infection prevalence of blood and spleen; (iii) all stages of the vector *I. ricinus* feed simultaneously on deer in large numbers during most of the year permitting transmission between tick instars during patent parasitaemias; (iv) *Ixodes ricinus* were found infected with *E. phagocytophila* at a site where the deer were also seropositive and where there were no sheep as alternative proven reservoirs (site 7); (v) and finally at a site (site 5) with a sparse population of *I. ricinus* the seroprevalence and infection prevalence in roe deer was lower than at a similar site with a dense population of *I. ricinus*.

Increasing numbers of deer in commercial conifer plantations may influence the epidemiology of TBF on adjacent sheep farms. However, we see no reason

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for regarding roe deer infected with *E. phagocytophila* as a threat to sheep farming. The management of TBF in endemic areas is based on ensuring exposure of lambs to natural infection from ticks to achieve endemic stability in the flock. Lambs, although susceptible to infection, acquire effective immunity whilst showing milder clinical reactions than older animals [34, 35]. However, ill-thrift and mortality in lambs have been recorded associated to tick pyemia caused by secondary staphylococcal septicaemia [36]. *Ixodes ricinus* in large areas of upland sheep farms have a patchy distribution [37], which will make the managerial maintenance of endemic stability more difficult. Increasing deer and associated tick populations may possibly lead to a decrease in patchiness of this distribution.

There is risk to humans of infection with tick borne ehrlichia in Europe although the prevalence is recorded as low [38, 39]. Additionally it has been suggested that granulocytic ehrlichia can be transmitted to humans by contact with infected deer blood [40]. The epidemiological relationship between the forms of the purported agent of HGE and of *E. phagocytophila* that occur in the United Kingdom urgently requires detailed clarification. Recent research suggests that the presence of deer near farms does not increase the burden of ticks or the prevalence of antibodies to granulocytic ehrlichia in humans [39]. In the absence of evidence of overt clinical disease of humans with the form of *E. phagocytophila* found in sheep and roe deer in the United Kingdom we see no reason for alarm but advocate continued vigilance for any change in the epidemiology of *E. phagocytophila* infection.

In this study we demonstrate that deer from populations with a high prevalence of *E. phagocytophila*-reactive antibodies also have the *E. phagocytophila groE* gene in their blood and spleen. We also have data to support site-specific geographic association between *I. ricinus* infestations and *E. phagocytophila* antibodies among wild roe deer populations. This study provides strong evidence to implicate roe deer as a competent reservoir for *E. phagocytophila* in the United Kingdom. Our data suggests that *E. phagocytophila* endemic cycles could be maintained in nature by a vector–reservoir host system consisting of *I. ricinus* and deer. This needs to be confirmed by transmission experiments using roe deer and with the development of long-term cultivation methods for *E. phagocytophila* that will enable the isolation of viable pathogens from the hosts.

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