REVIEW ARTICLE

Granulocytic ehrlichiosis: an emerging or rediscovered tick-borne disease?

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The Ehrlichiae are gram-negative obligately intracellular bacterial pathogens. They can be divided into at least three genogroups on the basis of 16S rRNA gene sequences, but are also classified by target cell specificity. A group of granulocytic ehrlichiae primarily infect neutrophils and fall into genogroup II. The granulocytic ehrlichiae are subdivided by their target hosts, i.e., *Ehrlichia phagocytophila* in cattle and sheep, *E. equi* in horses, and the agents of human (HGE) and llama (LGE) granulocytic ehrlichioses. However, these subdivisions may give a false impression, as all these species are closely related both antigenically and on the basis of 16S rRNA operon sequence. In addition, cross-species transmission can occur naturally or by experimental infection. The vectors for these granulocytic ehrlichiae are hard-bodied ixodid ticks, and the reservoir hosts are probably wild rodents, deer and sheep. In each host, this illness presents as a febrile disease which can be followed by immunosuppression leading to secondary infections.

Introduction

Members of the genus *Ehrlichia* have long been recognised as tick-borne, veterinary pathogens, but the recent emergence of two 'new' human pathogens, *E. chaffeensis* and the agent of human granulocytic ehrlichiosis (HGE), has rekindled interest in these organisms [1–3]. Clinical HGE infections occur in the USA and mainland Europe, and the causal organism belongs to the *Ehrlichia* genogroup II known as the granulocytic ehrlichiae. One member of this group, *E. (Cytoecetes) phagocytophila*, is common and widespread in the UK and mainland Europe. *E. phagocytophila* is closely related to or conspecific with the HGE agent, so considerable potential exists for human granulocytic ehrlichia infections to occur in the UK. Despite the widespread occurrence of the agents of granulocytic ehrlichioses – particularly *E. phagocytophila* whose importance as the causal agent of tick-borne fever (TBF) in domestic ruminants has been recognised for many decades – knowledge of these organisms and their epidemiology is rudimentary. Consequently, there is a renewed need for studies on the host associations, epidemiology and taxonomy of the granulocytic ehrlichiae and this article reviews current knowledge of these organisms as a basis for such studies.

Taxonomy

The genus *Ehrlichia*, named in 1945 in honour of Paul Ehrlich, comprises obligately intracellular, gram-negative bacteria which are classified in the α -subgroup of Protobacteria on the basis of sequence analysis of 16S rRNA genes [2–6]. Such sequence analysis differentiates the Ehrlichiae from the Rickettsiae and splits the Ehrlichiae into at least three genogroups (Table 1) [7–21]. The types of circulatory leucocytes targeted by each *Ehrlichia* species correspond, to some degree, with their genogroup. The major cellular targets of the ehrlichiae of genogroup I (e.g., *E. canis*, *E. chaffeensis*) and genogroup III (e.g., *E. risticii*, *E. sennetsu*) are monocytes and macrophages. Most species of genogroup II (e.g., *E. phagocytophila*, *E. equi* and the HGE agent) multiply mainly in neutrophils; those that do so are known as the 'granulocytic ehrlichiae' and the diseases they cause are called the granulocytic ehrlichioses.

The granulocytic ehrlichiae of the temperate zone infect domestic and wild animals and man, and are transmitted by hard-bodied (ixodid) tick vectors. Their widespread distribution in the USA, mainland Europe and the UK follows the geographic distribution of their vectors. *E. phagocytophila* was the first to be discovered, almost by accident, in 1932 [22]. This was followed by *E. (Cytoecetes) microti* in 1938, *E. equi* in 1969 [12], the HGE agent in 1994 [3] and, most

Table 1. *Ehrlichia* genogroups or clades: the bold surround indicates the temperate zone ‘granulocytic ehrlichiae’

Genogroup	Vertebrate host	Transmission	Vector	Target cells	Distribution
I					
<i>E. canis</i>	Canines	Tick bite	<i>Rhipicephalus sanguineus</i>	Monocyte, macrophage	USA
<i>E. chaffeensis</i>	Deer, man	Tick bite	<i>Amblyomma americanum</i> , <i>Dermacentor variabilis</i>	Monocyte, macrophage	USA
<i>E. ewingii</i>	Canines	Tick bite	<i>A. americanum</i>	Neutrophil	USA
<i>E. muris</i>	Mice, voles	Unknown	...	Monocyte, macrophage	USA
<i>Cowdria ruminantium</i>	Cattle, sheep, goats, antelope, buffalo	Tick bite	<i>Amblyomma</i> spp.	Endothelium	Tropics, worldwide
II					
<i>E. phagocytophila</i>	Sheep* [7], cattle [8], equines [9], dogs [10], deer [11]	Tick bite	<i>Ixodes ricinus</i> [7]	Neutrophil	Mainland Europe, UK
<i>E. equi</i>	Equines [12], dogs [13, 14] (experimentally; cats, primates [13])	Tick bite	<i>I. pacificus</i> [15], <i>I. ricinus</i> [16]	Neutrophil	USA, mainland Europe
<i>E. microti</i>	Voles* [17, 18]	Tick bite	<i>I. scapularis (dammini)</i> , [18]	Neutrophil	USA
HGE	Man* [3], deer [18, 19], rodents [18]	Tick bite	<i>I. scapularis</i> [20], <i>I. pacificus</i> [20], <i>I. ricinus</i> [21]	Neutrophil	USA, mainland Europe
LGE	Llama* [6]	Tick bite	<i>I. pacificus</i> [6]	Neutrophil	USA
<i>E. platys</i>	Canines	Unknown	...	Platelets	USA
<i>Anaplasma marginale</i>	Cattle	Tick bite	<i>Boophilus</i> spp.	Erythrocyte	USA
<i>A. ovis</i>	Sheep	Tick bite	<i>Rhipicephalus</i> spp., <i>D. andersoni</i>	Erythrocyte	USA
III					
<i>E. sennetsu</i>	Man	Ingestion of raw fish	...	Monocyte, macrophage	Far East
<i>E. risticii</i>	Equines, canines, felines	? Arthropods	...	Monocyte, enterocyte	USA
<i>Neorickettsia helminthoeca</i>	Canines	Ingestion	Salmon fluke	Macrophage	Worldwide
<i>N. elokominica</i>	Canines	Ingestion	Salmon fluke	Macrophage	Worldwide
<i>Stellantischnus falcatus</i>	?	?	Grey mullet fluke	?	?

*Species in which organism was first discovered.

recently, the agent of llama granulocytic ehrlichiosis (LGE) in 1997 [6]. Within the granulocytic *Ehrlichia* genogroup, temperate species have been named according to the vertebrate host in which they were first, or are commonly found (Table 1). However, a number of factors suggest strongly that such a classification may be misleading, and that these granulocytic ehrlichiae may even be the same species. Studies in Europe and the USA have demonstrated consistently that only 2–3-bp differences occur (approaching 100% homology) between the 1427-bp 16S rRNA sequences of *E. phagocytophila*, *E. equi* and the agents of HGE and LGE [2, 6, 14, 23, 24]. Furthermore, nucleotide sequences of the *groESL* heat shock operon of *E. phagocytophila*, *E. equi* and the HGE agent show 99–99.9% homology, with the deduced amino-acid sequences being identical [25]. What such small differences mean in terms of the vertebrate host associations of the granulocytic ehrlichiae is unclear. The 16S rRNA sequences of granulocytic ehrlichiae isolated from two different Swedish cattle (both thus ‘classified’ as *E. phagocytophila*) showed the same small degree of difference as that between 16S rRNA sequences of European isolates of *E. phagocytophila* and the HGE agent from the USA [23]. The biological behaviour of the granulocytic *Ehrlichia* ‘species’ is also remarkably similar in a number of respects. All appear to be transmissible to a wide, and similar, range of vertebrate species in similar geographic regions (Table 1). Experimental infections between species result in similar clinical consequences. For example, the HGE agent causes a clinical disease in horses that is indistinguishable from *E. equi* infection [26]. The HGE agent is serologically almost indistinguishable from *E. phagocytophila* and *E. equi*, the antigens of which are used to detect HGE in man [27, 28]. Furthermore, following recovery from experimental HGE infection, horses are protected against further challenge by *E. equi* [26].

Although the granulocytic ehrlichiae may comprise a single species, or are at least indistinguishable on the basis of host association, strain variation certainly occurs. Isolates of *E. phagocytophila* from cattle and sheep in different geographic foci of infection within the same country vary considerably with regard to their virulence and ability to cross-protect [29, 30]. Antigenic variation has been observed also in isolates of the HGE agent [31]. Clearly, further studies are required to investigate the taxonomy of the granulocytic ehrlichiae and how this relates to their antigenic heterogeneity, virulence and vertebrate host associations.

The vector

The ixodid (hard-bodied) tick *Ixodes ricinus* is the vector of *E. phagocytophila*, and is likely to be the

vector of *E. equi* and HGE in Europe [7, 16, 21]. In central and eastern USA, *I. scapularis* is the vector of HGE and, in the west, *I. pacificus* is the vector of *E. equi* [20]. All these tick species will feed on almost any terrestrial vertebrate, which is why they are important vectors of tick-borne zoonoses in Europe (including the UK) and the USA [32]. The ticks have three feeding stages (larva, nymph and adult) which are parasitic only for short periods. Each stage feeds once only, for 3–14 days, and then randomly drops off the host into the herbage litter layer. Here they moult to the next developmental stage and may remain for up to 1 year before becoming active, whereupon they ascend the herbage to wait for another host and their next meal.

In order to transmit infection from one host to another, granulocytic ehrlichiae must survive in the tick through the moulting process (known as transstadial transmission). *E. phagocytophila* is maintained transstadially, and ticks acquiring infection as larvae may be infective as both nymphs and adults [7]. Vertical transmission from an adult female to her offspring larvae (transovarial transmission) occurs in some tick-transmitted infections, but such transmission of *E. phagocytophila* either does not occur or does so only with low efficiency [7, 33]. The development and growth of granulocytic ehrlichiae in the vector has not been studied, but it is likely that, as with other tick-borne rickettsias, multiplication occurs within a number of tissues, including the salivary glands, and that the organism is transmitted to the vertebrate host in the saliva of the feeding tick [34]. Some processing of the organism within the tick may have to occur during feeding before the organism becomes infective, because macerates of infected, unfed ticks are not infective to sheep, whereas those prepared from ticks that have been allowed to partially feed on an uninfected host are infective [33].

The risk of infection to man and domestic animals depends on what proportion of questing ticks in a particular focus are infected and on the density of actively questing ticks. The density of active ticks is a function of their seasonal activity. For example, in upland regions of the UK, the main peak of *I. ricinus* tick activity occurs in spring and early summer, with a lesser peak in autumn, while ticks in woodlands may quest continuously, reaching a peak of activity in mid-summer [35]. Consequently, animal and, if they occur in the UK, human infections are most likely to present to clinicians during these peaks of tick activity.

Reservoirs

The question of which species are the most important reservoirs of the granulocytic ehrlichiae remains unresolved. The tick vectors will feed on almost any terrestrial vertebrate, which explains why a single

granulocytic *Ehrlichia* species can cause infections in a diverse range of vertebrate species. However, for an infected animal to be a 'competent' reservoir requires that it then transmits infection to uninfected ticks which maintain the infection trans-stadially and, subsequently, infect another vertebrate. So far, only sheep have been conclusively demonstrated to be 'competent' reservoirs [7], but any species that can be infected with granulocytic ehrlichiae could be a potential competent reservoir. The importance of any one reservoir species in maintaining endemic cycles of granulocytic ehrlichia infection depends on the proportion of ticks that feed on that species. In woodlands, the natural habitat for *I. ricinus* and *I. scapularis*, a wide variety of wild mammals and birds feed and maintain the ticks. Deer are important hosts of ticks in woodlands and may be reservoirs of *E. phagocytophila* and the HGE agent [11, 19]. Wild woodland rodents also feed many ticks and, even though laboratory rodents are resistant to granulocytic ehrlichia infection [29, 36], their wild cousins may be important reservoirs [18]. Studies in our laboratory suggest that cycles of *E. phagocytophila* infection are maintained in UK woodlands by wild species. However, by increasing the density of domestic livestock, man's influence can result in these species becoming the dominant tick hosts. Sheep on many UK uplands host nearly all *I. ricinus* ticks of all three feeding stages [37] and, being competent reservoirs, are likely to be crucial to maintenance of *E. phagocytophila* in these habitats.

In contrast, dogs, well-groomed horses kept for recreation and man are unlikely to be important reservoirs because they occur at low densities in the countryside and feed few ticks. Any ticks that do attach to these hosts are unlikely to survive to find and infect another susceptible host. For these reasons, man, dogs, recreational horses and, possibly, pet llamas in California [6] are most likely to acquire granulocytic ehrlichia infections by accident from ticks that became infected while feeding on wild species or domestic livestock.

Transmission efficiencies have not been quantified from either vertebrate host to tick, or tick to host, but it may take only one *E. phagocytophila*-infected *I. ricinus* tick to provide a sufficient infective dose to produce clinical disease (i.e., TBF) in a naïve sheep [33]. Following infection, sheep remain infective to ticks for at least 35 days, and possibly as long as 2 years, so a high prevalence of infection in questing nymphal and adult ticks could be expected in UK uplands. Indeed, the probability that a susceptible sheep will acquire infection on tick-infested pasture is probably 100%—a feature recognised by farmers of tick-infested UK uplands who will only buy sheep that have been exposed previously to ticks and are likely to have some immunity to clinical TBF. The consequences of failing to observe this precaution can be catastrophic, as demonstrated when 225 (91%)

of 280 naïve, pregnant sheep aborted, because of *E. phagocytophila* infection, within 4 weeks of introduction to *I. ricinus*-infested pasture [38], including 25% of these animals that aborted within 9 days of introduction to the pasture.

Artificial culture

Only recently has pure growth of the granulocytic ehrlichiae been achieved in artificial culture. *E. equi* and the HGE agent have both been grown in the human promyelocytic leukaemia cell line HL60 [39], where both form similar intravacuolar microcolonies (morulae) that differ greatly from those formed by ehrlichiae of other genogroups [40]. *E. equi* has been cultured in a tick-cell line [41]. It remains to be seen whether *E. phagocytophila* may be cultured similarly. To date, infected sheep have been the main source of this bacterium.

Pathogenesis and pathology

To choose to grow in a professional phagocyte such as the polymorphonuclear neutrophil is analogous to putting one's head in a lion's mouth to scavenge food from between its teeth. This problem is compounded by the short (6–12 h) life-span of the neutrophil.

Most of our understanding of the pathogenesis of the granulocytic ehrlichioses is based on studies of *E. phagocytophila* infection of sheep, which does, however, display some marked similarities to HGE infection in man. Following experimental inoculation or feeding of an infected tick, *E. phagocytophila* can be detected microscopically in circulating leucocytes after 3 days. However, blood is infective to other sheep within 24 h of inoculation [42]. Cytoplasmic inclusions appear first in eosinophils, then neutrophils, and finally monocytes, over a period of 7 days, and inclusions may be detected in neutrophils for 2–3 weeks after infection [43]. *E. phagocytophila* enters and multiplies in polymorphonuclear cells of the peripheral circulation [44] but, following experimental infection, has also been detected in alveolar macrophages and Kupffer cells [45]. *E. phagocytophila* enters host cells by endocytosis and is found within endocytic vacuoles [46] containing one or more organisms (Fig. 1). Larger inclusions which contain dividing bacteria are called morulae (Fig. 2). It appears that there is inhibition of phagolysosome fusion (unpublished observations); thus the bacteria may not be exposed to lysosomal enzymes and may also evade the neutrophil's oxidative burst. Similar changes and inclusions have been described for *E. equi* and HGE infections [40]. The bacteria are released from neutrophils by unknown mechanisms, but they may be found in the plasma of infected sheep, which is in turn infective [47]. Granulocytic

Fig. 1. Thin-section electronmicrograph of an ovine neutrophil showing an endocytotic vacuole containing *E. phagocytophila*.

Fig. 2. Thin-section electronmicrograph of an ovine neutrophil containing growing *E. phagocytophila* within an endocytotic vacuole (morula).

ehrlichioses in all species are accompanied by pyrexia and immunosuppression. TBF in sheep is characterised by severe leucopenia associated with early lymphocytopenia (of B lymphocytes and CD4+, CD8+, but not γ - δ T lymphocytes) and prolonged neutropenia that compromises the immune response. In addition, lymphocytes of infected sheep have reduced responses to mitogens, and neutrophil attachment, phagocytosis and killing are inhibited [48–50]. Granulocytic ehrlichiae may also infect thrombocyte precursors in bone marrow, as thrombocytopenia has been observed in *E. phagocytophila*, *E. equi* and HGE infections [28, 51, 52]. In addition, granulocytic ehrlichiae may infect endothelial cells in horses [51].

Clinical features

There are striking similarities in the clinical features of the granulocytic ehrlichioses of domestic animals and man. Primary infections result in pyrexia and immunosuppression, which may render the host susceptible to more serious and potentially fatal secondary infections.

Granulocytic ehrlichiosis in sheep and cattle

Pyrexia is the first sign of infection and follows an incubation period of 3–4 days. It is maximal on the second day of parasitaemia, but the duration and magnitude vary between isolates of *E. phagocytophila* [53, 54]. Affected animals are dull and anorexic, a rapid fall in milk production occurs in dairy cattle and, if pregnant, cattle and sheep usually abort [55, 56]. Infertility associated with spermatopenia and abnormalities of spermatozoa occurs in infected males [57]. The most important clinical consequences are a result of secondary infections, the most common of which in sheep is ‘tick pyaemia’ caused by secondary staphylococcal septicaemia. Multiple abscesses develop in almost any tissue, resulting in ill-thrift, crippling lameness and death. An estimated 300 000 lambs suffer tick pyaemia every year in UK uplands, which represents 1% of the total UK sheep flock [58]. *E. phagocytophila* infection renders both cattle and sheep more susceptible to respiratory tract infections with *Pasteurella* spp. and *Chlamydia psittaci* [54]. Sheep are rendered susceptible to clostridial diseases and the pathogenicity of the tick-borne flavivirus causing ‘louping ill’ is enhanced. Cattle may be rendered susceptible to Johnes disease (caused by *Mycobacterium paratuberculosis*) and to listeriosis [54].

Granulocytic ehrlichiosis in man

Most described cases have occurred in Wisconsin and Minnesota, USA [28], but human infection has been detected in Slovenia [24] and Sweden [59]. Following an infective tick bite, an incubation period of 1–60 days (median 8 days) elapses before the onset of pyrexia (mean 39.3°C). Accompanying symptoms include

myalgia, headache, nausea, rigors and arthralgia, but infection with granulocytic ehrlichiae alone may be short-lived and self limiting [24, 28]. In one study of 41 affected patients, 23% were hospitalised and three were admitted to intensive care, of whom two later died. Twelve patients suffered respiratory symptoms, some with radiographically detectable pneumonia. Both patients who died suffered opportunist or secondary infections; *Candida albicans* pneumonia, *Cryptococcus neoformans* pneumonia and gastro-oesophageal haemorrhage caused by herpes simplex virus 1 and *C. albicans* [28]. HGE has, thus far, been diagnosed more frequently in males, those aged >40 years, and those with regular contact with animals [28]. Recent evidence suggests that patients infected with the HGE agent may frequently be co-infected with the tick-borne pathogens *Borrelia burgdorferi* s.l. (the Lyme disease spirochaete) and *Babesia microti*. HGE may alter or enhance the pathogenicity of these infections [60].

Granulocytic ehrlichiosis in horses and llamas

Subclinical infection or infection accompanied by mild clinical signs may be the most frequent outcome of infection in horses. The prevalence of anti-*E. equi* antibodies far outweighs that of detected clinical disease [61]. Clinically affected equines and llamas are pyrexemic and show vague symptoms of depression, anorexia and slight hind-limb ataxia [6, 9, 51]. Some horses develop limb oedema and cardiac arrhythmias associated with vasculitis, but most recover within 7–14 days [51].

Diagnosis and treatment

HGE should be considered as a differential diagnosis for previously healthy individuals who present with ‘flu-like’ symptoms following outdoor activity in the USA or mainland Europe where they may have been exposed to tick bites. It may also be advisable to consider HGE as a possible cause of similar symptoms in patients with a history of tick bite in the UK. Diagnostic adjuncts include peripheral white blood and platelet counts, the demonstration of morulae in neutrophils and raised C-reactive protein [28]. Serological diagnosis is possible with *E. equi* or *E. phagocytophila* antigens [27, 28], but definitive diagnosis is by detection of granulocytic *Ehrlichia* 16S rRNA gene DNA by a specific PCR [2, 3, 6, 14, 23, 24] or by direct culture [39, 41]. Tetracycline or deoxycycline are the antimicrobial agents of choice and prolonged administration (up to 2 weeks) may be necessary to avoid relapses.

Conclusions

Although HGE has just emerged, as with most ‘new’ diseases this probably represents a lack of recognition

rather than an entirely new disease. Clearly, the granulocytic ehrlichiae of wild and domestic animals have been present for many years, and it is likely that it is these infections which, via tick-borne transmission, result in HGE infections. The current taxonomy of the granulocytic ehrlichiae, based on the vertebrate species in which they were first discovered, is likely to be misleading because of the catholic feeding behaviour of the tick vectors. The granulocytic ehrlichiae may well all belong to the same species, yet variations between isolates do occur. How such variations may relate to adaptations to different reservoir host species, variations in virulence in domestic animals and man, and to different geographic distributions awaits a re-evaluation of their taxonomy. These bacteria also pose fascinating questions. How do they survive and multiply in phagocytic cells? Why does an organism with such a wide geographic distribution and a wide host range show a relative lack of genetic diversity? While answers to these questions are awaited, the granulocytic ehrlichiae continue to pose major health and welfare problems for domestic animals, affect food production and pose a risk to humans health.

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