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Dissecting the Enzootic Cycles of *Anaplasma phagocytophilum* in a Multi-host, Multi Vector World

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Anaplasma phagocytophilum has long been recognised as the pathogen of veterinary importance, primarily causing tick-borne fever in sheep and cattle, but also being associated with infections in other domesticated animals including cats, dogs and horses. However, more recently it has emerged as a zoonotic agent, causing human granulocytic anaplasmosis (HGA). Following its first description in the USA just over a decade ago, HGA has been reported across Europe and North and South America, and in the USA at least, HGA is now recognised among the most medically important tick-borne diseases.

A. phagocytophilum is transmitted by ticks of the genus *Ixodes* and is thought to exploit a wide range of mammals as reservoir hosts. In Europe, *Ixodes ricinus*, which has a broad host range, is generally considered the most important vector for *A. phagocytophilum*; surveys of questing ticks belonging to this species collected across the continent have, in some locales, revealed the presence of the bacterium at a prevalence of over 20%. However maintenance of *A. phagocytophilum* in places where *I. ricinus* is absent indicates that other *Ixodes* species are also likely competent vectors. The ability of different *Ixodes* species to transmit *A. phagocytophilum* is also well recognised in North America, where *Ixodes scapularis* and *Ixodes pacificus* are important vectors on the Eastern and Western sides of the continent respectively. In Eastern Asia, *A. phagocytophilum* has been detected in questing *Ixodes persulcatus* and *Ixodes ovatus* ticks. As mentioned above, in addition to an ability to exploit different *Ixodes* species as vectors, *A. phagocytophilum* is thought to be capable of exploiting a wide range of wildlife species as reservoir hosts. Apparently asymptomatic infections have been detected in various mammal species, most frequently cervids and rodents, in Asia, Europe and North America.

In the United Kingdom, as elsewhere, the three life stages of *I. ricinus* demonstrate clear host preferences. Larval ticks feed mainly on rodents whereas nymph and adult stages feed on larger mammals such as squirrels and, particularly, deer. This specificity has important implications for the natural persistence of *A. phagocytophilum*; unfed larval ticks cannot be infected with *A. phagocytophilum* as infections are not transmitted transovarially by infected female ticks to their offspring. Thus, the rodent hosts on which these larvae eventually feed cannot become infected and hence will not be infectious to other ticks that feed upon them. Due to these circumstances, rodents have been largely discounted as important reservoir hosts for *A. phagocytophilum*.

However, as discussed above, tick species other than *I. ricinus* are also competent vectors for *A. phagocytophilum*, and we have hypothesised that the co-existence of such species with *I. ricinus* will have significant repercussions for *A. phagocytophilum* transmission. We have tested this hypothesis by focusing on *Ixodes trianguliceps*, all three life stages of which feed exclusively on small mammals, with the assumption that should this species be a competent vector of *A. phagocytophilum*, then rodents may have a far more important role in the natural maintenance of the pathogen than previously recognised.

Through a combination of capture-mark-recapture field studies and molecular diagnostics, we have been able to monitor and quantify *A. phagocytophilum* infections in, and tick burdens on, woodland rodent (*Apodemus sylvaticus* and *Clethrionomys glareolus*) populations inhabiting the Wirral, a region of North West England where (unusually) *I. ricinus* is absent. This study resulted in the demonstration that *A. phagocytophilum* could be maintained in an *I. ricinus*-free enzootic cycle likely comprising of solely woodland rodents as hosts and *I. trianguliceps* as vectors.

Subsequently we have tested our hypothesis by asking if, in locations where both *I. trianguliceps* and *I. ricinus* co-exist, *A. phagocytophilum* can be maintained in a *I. trianguliceps*-rodent enzootic cycle and can also be transmitted out of this cycle by *I. ricinus* larvae feeding on infected rodents. Given the almost ubiquitous distribution of *I. trianguliceps* in the UK and widespread distribution of *I. ricinus*, particularly in upland areas of the country, co-existence of these two species is undoubtedly frequent. Using field studies in Northumberland, close to the Scottish border, we have spent 3 years monitoring the population dynamics of grassland rodents (mainly *Microtus agrestis*), feeding *I. trianguliceps* and feeding and questing *I. ricinus*, and the *A. phagocytophilum* infection status of hosts and vectors. We have been able to demonstrate that, as on the Wirral, rodents are frequently infected with *A. phagocytophilum* and that even in the presence of *I. ricinus*, the enzootic cycle involving rodents and *I. trianguliceps* remains intact and viable. We have also demonstrated another enzootic cycle involving, as expected, *I. ricinus* and deer (*Capreolus capreolus*), thereby provoking the key question as to whether rodent-feeding *I. ricinus* larvae serve as a "bridge" between these two cycles and thus implicating rodents as important players in the transmission of *A. phagocytophilum*. In order to address this question, we have recently been developing and employing sensitive molecular typing methodologies, hypothesising that if the species is maintained in different enzootic cycles, one of the consequences of these ecological differences should be the existence of measurable diversity among *A. phagocytophilum* strains. The results of these efforts in the context of dissecting *A. phagocytophilum* enzootic cycles in the UK will be discussed.