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European human granulocytic anaplasmosis is caused by a subcluster of *Anaplasma phagocytophilum* Ecotype I

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ABSTRACT

Anaplasma phagocytophilum causes human granulocytic anaplasmosis. However, despite its ubiquitous presence in animals and ticks, human cases are rarely reported in Europe. We generated genetic data from *A. phagocytophilum* from patients and compared them with sequences from wild and domestic animals to assess the zoonotic potential of the respective genotypes. The genomic sequence of an *A. phagocytophilum* isolate obtained from a Slovenian patient was determined. We also sequenced a *groEL*-gene fragment of eight isolates from human patients from France and Poland. The *A. phagocytophilum* genome from the Slovenian patient was more closely related to isolates from dogs than from sheep. Using *groEL*-based typing, isolates from humans were found within a distinct subcluster of *A. phagocytophilum* Ecotype I. This subcluster was defined as zoonotic. Strains from dogs, horses, cats, foxes, wolves, and wild boar were significantly overrepresented in this branch. Variants outside this subcluster were more abundant and found in a wider variety of domestic and wild animals, most notably ruminants. A similar pattern was observed for the MLST analyses targeting seven housekeeping genes. Human anaplasmosis in Europe is associated with a specific subcluster of *A. phagocytophilum* Ecotype I, which is not primarily associated with ruminants, but rather with dogs, horses, cats, carnivores, wild boar and hedgehogs. Our findings provide a reasonable explanation for the discrepancy between the omnipresence of *A. phagocytophilum* in the environment and the limited number of reported human cases. We recommend taking this genetic sub-clustering into account for future risk assessments.

1. Introduction

The incidence of tick-borne diseases is expected to rise, driven by a combination of human demographics and environmental and climatic changes (Lindgren et al., 2012; Medlock et al., 2013; Köhler et al., 2023). In Europe, *Ixodes ricinus* is widely distributed, particularly in the

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central and northern regions of the continent (Černý et al., 2020). Here, it is the primary vector of *Borrelia burgdorferi* (*sensu lato*), the causative agent of Lyme borreliosis, and of tick-borne encephalitis virus, which together account for the two most common tick-borne diseases in most countries of Europe (Černý et al., 2020). In addition, other *I. ricinus*-borne pathogens such as *Anaplasma phagocytophilum*, *Borrelia miyamotoi* and *Neoehrlichia mikurensis* appear to be emerging as well (Sprong et al., 2018).

Anaplasma phagocytophilum causes febrile disease in humans and animals called granulocytic anaplasmosis (Matei et al., 2019). Concerning human granulocytic anaplasmosis (HGA), significant variation in disease epidemiology is observed. Whereas HGA is common in the USA, the number of published cases in Europe is very low (Matei et al., 2019; Azagi et al., 2020; Dixon et al., 2021). This is remarkable, as *A. phagocytophilum* is ubiquitously present in ticks as well as in various domestic and wild animals across Europe (Stuen et al., 2013; Matei et al., 2019).

There are multiple possible explanations for the low number of human cases in Europe despite the widespread distribution of the pathogen in ticks and animals. The disease might be underreported as it is not a notifiable disease in most European countries. It might be underdiagnosed as it presents itself with non-characteristic symptoms such as fever, chills, malaise, headache, arthralgias and myalgias (Ismail and McBride, 2017). This is reinforced by a presumably low awareness among health professionals and the lack of laboratory diagnostics such as PCR or serological tests in routine settings. Lastly, not all variants of *A. phagocytophilum* which circulate between ticks and animals might be zoonotic. The latter is investigated in this study.

Despite the increasing number of studies on the genetic diversity of *A. phagocytophilum*, our understanding of the zoonotic potential of the genetic variants remains poor. *Anaplasma phagocytophilum* is currently regarded as a single species. However, various genetic markers, often very short or highly variable, indicate that *A. phagocytophilum* can be subdivided into groups, which differ in their host tropism and probably in their transmission cycle and geographical origin (Jaarsma et al., 2019; Langenwalder et al., 2020a; Rar et al., 2021).

The *groEL* gene encodes a heat-shock protein, and sequence information is available for more than 1600 *A. phagocytophilum* isolates. Sequence variation within that gene has been successfully used to infer information about spatial segregation and associations of genotypes to particular vector-host interactions. Our studies on *groEL*-based typing identified four ecotypes of *A. phagocytophilum* in Europe (Jahfari et al., 2014). Human isolates from Europe clustered only within Ecotype I (Jahfari et al., 2014; Jaarsma et al., 2019), which is therefore considered zoonotic. Ecotype I has the widest host range among the four ecotypes. It is found in domestic animals and wildlife such as red deer, carnivores, hedgehogs and wild boar (Jahfari et al., 2014; Jaarsma et al., 2019). Previous studies suggested that only a fraction of genetic variants within Ecotype I might have zoonotic potential (Jaarsma et al., 2019; Hrazdilová et al., 2021). Ecotype II is detected in roe deer and only sporadically in other mammalian species, Ecotype III is linked to small mammals, especially rodents, whereas Ecotype IV is found mainly in birds (Jahfari et al., 2014).

The *groEL*-based typing allows, with reasonable effort, the characterization of many *A. phagocytophilum* strains because a short fragment is used. On the other hand, the *groEL* marker may lack the resolution to explain the discrepancy between the widespread presence of *A. phagocytophilum* in the environment and the low number of human cases in Europe. The analysis based on whole-genome sequencing (WGS) of isolates should overcome this limitation. However, the only four complete genomes from Europe available in the GenBank database were derived from animals (as of August 2025). This is due to the fact that the European variants of *A. phagocytophilum* are extremely difficult to isolate and cultivate.

Here, we describe the first *A. phagocytophilum* genome from a European HGA patient and compare it with the four European genomes obtained from domestic animals and other complete genomes of *A. phagocytophilum* from the USA and Asia. Together with data from *groEL*-based typing and multilocus sequence typing (MLST), we infer that all *A. phagocytophilum* isolates from European HGA patients form a small group within Ecotype I and MLST Cluster 1, which is associated with a restricted number of animal species.

2. Materials and methods

2.1. Whole genome sequencing, assembly, annotation and comparison

The human isolate of *A. phagocytophilum,* herein referred as SLO-1, was obtained from a HGA patient (Supplementary file 1). The cultivation of strain SLO-1 in HL60 cells (Supplementary file 1) was done essentially as described (Carlyon, 2005). For whole genome sequencing, 1.7×10^7 infected HL-60 cells (28th strain passage, infection rate 80%) were pelleted. Their DNA was extracted with the QIAamp DNA mini kit (Qiagen, Hilden, Germany). DNA quality was measured *via* electrophoresis using the Genomic DNA ScreenTape Analysis on an Agilent 4200 TapeStation System (Agilent Technologies Netherlands BV, Amstelveen, The Netherlands). The amount of DNA was quantified using the Qubit dsDNA HS Assay Kit on a Qubit 3.0 Fluorometer (Life Technologies Europe BV, Bleiswijk, The Netherlands). The DNA was then used to prepare 1D ligation libraries applying the Ligation Sequencing Kit SQK-LSK110 (Oxford Nanopore Technologies, Oxford, UK). The library was sequenced on a FLO-MIN106 flow cell using Guppy v.6.3.2 at super accuracy for base calling (Oxford Nanopore Technologies). Contigs were *de novo* assembled from the unaligned reads using Flye 2.9.1-b1780 and further polished with Medaka v1.4.3. The polished genome sequences were annotated *via* Prokka v1.14.6. BUSCO v5.2.2 was used for quality control of the annotated genome sequences based on the rickettsiales_odb10 lineage dataset. The obtained genome of strain SLO-1 was compared to fully assembled *A. phagocytophilum* genomes using the Anvi'o pangenomic workflow (version 7) with the MCL inflation parameter set to 2 (Eren et al., 2021). While GenBank currently lists 31 *A. phagocytophilum* genome entries, many of these are draft assemblies with multiple contigs, variable annotation quality, and potentially missing loci. Therefore, we selected twelve high-quality genomes each assembled into a single scaffold and available as of January 2024 from the European Nucleotide Archive (ENA). These genomes represent isolates from the USA, Europe, and Asia (Supplementary file 2). The Anvi'o genome databases were annotated using the NCBI COG function (Galperin et al., 2015). The JSpecies Web Server was used for calculating average nucleotide identity (ANIb) based on BLAST+ (Richter et al., 2016). Single-copy core genes (SCG) were identified using the Anvi'o (version 7), and the gene alignment from SCG was extracted and concatenated using the program "anvi-get-sequences-for-gene-clusters", with additional commands: –concatenate-gene-cluster; –report-DNA-sequences. The Shimodaira-Hasegawa-like approximate likelihood ratio test (SH-aLRT) and the ultrafast bootstrap approximation (UFB) were used to calculate bootstrap values with the same software. The subsequent phylogenetic analyses were computed using IQ-tree (Nguyen et al., 2015) and visualised and edited in FigTree (v1.4.1).

2.2. Multilocus sequence typing

MLST data were retrieved from the *A. phagocytophilum* MLST database hosted on PubMLST (https://pubmlst.org/aphagocytophilum/). The seven housekeeping genes (*atpA*, *dnaN*, *fumC*, *glyA*, *mdh*, *pheS*, and *sucA*) were concatenated (2877 bp) in the same order as previously described (Rar et al., 2021) and aligned by Clustal Omega using the Geneious Prime software v2025.0.3 (as of January 2025). A total of 391 MLST isolates from 17 host species and 12 European countries representing MLST Cluster 1, which corresponds mostly to the *groEL* Ecotype I, were selected. The MLST profile of strain SLO-1 has been reported

before as "human_Slovenia" (Langenwalder et al., 2020a).

2.3. groEL-based typing

A collection of 1623 *groEL* gene fragments (Jaarsma et al., 2019) including information on host species and country of origin, was updated with 1661 additional sequences retrieved from GenBank (as of January 2025). Isolates with nucleotide sequences shorter than 366 bp were excluded. Sequences were aligned using Clustal Omega within Geneious Prime software v2025.0.3. Only sequences that corresponded to the 366-bp fragment of the *groEL* gene (Jahfari et al., 2014) were used in subsequent analyses. Duplicates were removed according to the accession number, followed by the exclusion of sequences not belonging to Ecotype I. The 366 bp fragment of the *groEL* gene was amplified and sequenced from samples from five French (Jaulhac, unpublished observation) and three Polish HGA patients (Moniuszko-Malinowska et al., 2021) as previously described (Jahfari et al., 2014). The final dataset comprised 1054 *groEL* fragments of Ecotype I. These eight patients have not travelled abroad in the weeks before the onset of the disease and are considered autochthonous cases.

2.4. Network analyses

The relationship between *A. phagocytophilum* haplotypes was estimated for the MLST and *groEL* gene datasets described above. Median-joining network analyses were calculated for 391 concatenated MLST sequences (2877 bp) belonging to MLST Cluster 1 and for 1054 *groEL* gene fragments (366 bp) of Ecotype I using PopART (Leigh et al., 2015) on the multiple sequence alignments generated with the Geneious Prime software v2025.0.3.

To analyse whether the probability that *A. phagocytophilum* genotypes belong to the zoonotic, rather than to the non-zoonotic variant, differs from the overall background (all other species combined), Fisher's exact test (Fisher, 1935) was used independently for each host species. A zoonotic variant was defined as an *A. phagocytophilum* isolate which was genetically highly similar to isolates found in humans. The test was chosen due to the binary nature of the groups and the small sample sizes. Since multiple comparisons were conducted, the Benjamini-Hochberg procedure was applied to control for the false discovery rate (FDR) (Benjamini and Hochberg, 1995). The association of host species with a zoonotic variant was considered statistically significant if its adjusted FDR *P*-value was less than 0.05.

To analyse whether the distribution of zoonotic *versus* non-zoonotic strains is better explained by host species or by country of isolate origin, we fitted two binomial logistic regression models. The first model included host species as a categorical predictor, while the second one included the country of origin. Model performance was compared using the Akaike Information Criterion (AIC) to assess relative fit. McFadden's pseudo-R^2 was applied to estimate the proportion of variance explained (McFadden, 1974). All statistical analyses were performed in R v4.2.2 (https://www.r-project.org/).

3. Results

3.1. Genome assemblies of A. phagocytophilum

A complete circular genome of *A. phagocytophilum* was obtained from the isolate of the Slovenian patient (Lotric-Furlan et al., 2004) and is further referred to as SLO-1. The genome assembly contains 1282 coding sequences (CDS), 37 transfer RNAs, 3 ribosomal RNAs, has a size of 1, 517,188 bp and a guanine-cytosine base content (GC) of 41.7%. The assembly is characterised by a high BUSCO score value (98.4%) and is represented in one scaffold (Supplementary file 2).

3.2. Genome comparison

The phylogenetic analysis of twelve selected complete genomes of *A. phagocytophilum* (Supplementary file 2) and SLO-1 revealed the presence of three clusters based on geographical origin (Fig. 1). We used the Average Nucleotide Identities (ANI) to further investigate genomic relationships between *A. phagocytophilum* genotypes. Previous analyses of genome databases questioned the existence of a microbial species boundary and the proposal that the cut-off for it could be uniformly set at 95% ANI (Murray et al., 2021). The results of ANI calculation for the 13 *A. phagocytophilum* isolates resulted in a maximum ANI of 99.97% and a minimum ANI of 93.09% (Supplementary file 2). The SLO-1 genome was more similar to the four isolates from Europe than to the human isolates from the USA and South Korea (Supplementary file 2). The similarity was highest to the Austrian dog isolate, followed by the Dutch dog isolate and the two Norwegian sheep strains (Fig. 1).

3.3. MLST analysis

In total, 391 isolates belonging to MLST Cluster 1 from 12 European countries were included: 35 human isolates from Slovenia ($n = 33$) and Poland ($n = 2$), 242 isolates from domestic animals, 70 from wildlife and 44 from ticks (Table 1). Phylogenetic clustering revealed that all human sequence types (ST 25, 54, 55, and 162) branched together with STs from dogs, horses, cats, wild boar, foxes, hedgehogs, three sheep, one goat and *I. ricinus* ticks (Fig. 2, Supplementary file 2, Supplementary file 3: Fig. S1) as published previously (Huhn et al., 2014; Langenwalder et al., 2020a). In this branch, isolates from dogs, horses, cats, hedgehogs and wild boar were significantly overrepresented, whereas cattle, sheep and red deer were significantly underrepresented (Table 1). These data suggest that the STs from this branch can cause HGA and are infectious to dogs, horses, cats, hedgehogs and wild boar as reported previously (Huhn et al., 2014; Langenwalder et al., 2020a). The *A. phagocytophilum* STs of MLST Cluster 1 most frequently found in ruminants (cattle, sheep and red deer) were not associated with STs causing HGA. Although the majority of the isolates from the zoonotic branch originated from Germany ($n = 134$), Slovenia ($n = 54$) and Switzerland ($n = 18$), this branch also contained isolates from Austria, Denmark, Finland, France, the Netherlands, Poland, Spain, and Sweden. The model including host species as a predictor, provided a substantially better fit than the model based on the country of origin. Specifically, the host model yielded a lower AIC (152.38) and a higher McFadden's pseudo-R^2 (0.776), indicating that it explains a larger proportion of the variance and is a much stronger predictor of zoonotic potential. In contrast, the country model had a higher AIC (351.67) and a lower McFadden's pseudo-R^2 (0.381).

3.4. The groEL gene analysis

In total 1054 isolates belonging to *A. phagocytophilum groEL* Ecotype I from 25 European countries were used in the analyses: 11 human isolates from France ($n = 5$), Poland ($n = 3$), Slovenia ($n = 2$) and the Netherlands ($n = 1$), 296 isolates from domestic animals, 387 from wildlife and 360 from ticks (Table 2). Phylogenetic clustering revealed that all human haplotypes branched together (Fig. 2, Supplementary file 2, Supplementary file 4: Fig. S2). The majority of the isolates in the zoonotic branch (Supplementary file 4: Fig. S2) were from dogs (22%, 28/128), foxes (15%, 19/128), horses (13%, 16/128), wild boar (10%, 13/128), and cats (9%, 12/128). In this branch, isolates from dogs, horses, cats, foxes, wolves and wild boar were significantly overrepresented whereas cattle, sheep and red deer were significantly underrepresented (Table 2). Haplotypes from the zoonotic branch were found in 17 of the 25 countries indicating that the zoonotic variant is widespread and not restricted to the four countries with HGA patients. As in the MLST dataset, the model including host species as a predictor provided a substantially better fit than the model based on country of origin. Specifically, the host model yielded a lower AIC (410.68) and a

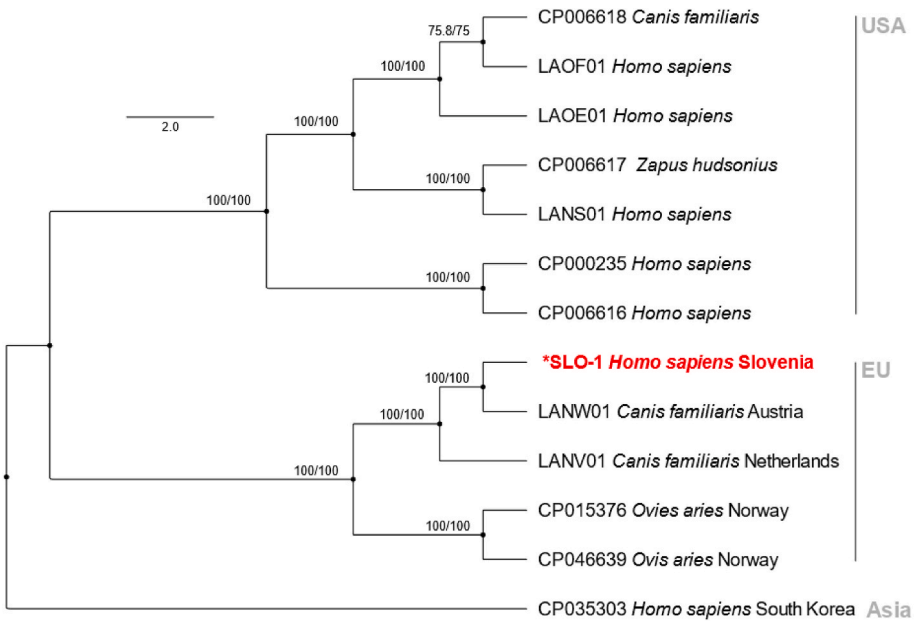

Fig. 1. The maximum likelihood phylogenetic tree was constructed based on the single-copy core genes (SCGs) of the 13 *A. phagocytophilum* genomes (Supplementary file 2). The *A. phagocytophilum* genome from a human from Slovenia (SLO-1) is marked in red. The bootstrap values are displayed near the branches. The scale bar represents the number of substitutions per site. *Abbreviation*: EU, Europe.

Table 1
European *A. phagocytophilum* MLST Cluster 1 strains are divided in zoonotic and non-zoonotic branches, which are inferred from Fig. 2. For more details, see Supplementary file 2.

Host species	Non-zoonotic	Zoonotic	Country	Odds ratio	*P*-value
Bison bonasus	6	0	1	Inf	0.062
Bos taurus	57	0	4	Inf	**< 0.0001**
Canis lupus familiaris	0	75	9	0	**< 0.0001**
Capra aegagrus hircus	2	1	2	1.488145	1
Capra ibex	2	0	1	Inf	0.612
Cervus elaphus	8	0	4	Inf	**0.023**
Cervus nippon	5	0	1	Inf	0.105
Dama dama	1	1	1	0.74335	1
Equus caballus	4	43	5	0.06325	**< 0.0001**
Erinaceus europaeus	0	24	1	0	**< 0.0001**
Felis catus	1	13	5	0.055781	**< 0.001**
Homo sapiens	0	35	2	0	**< 0.0001**
Ixodes ricinus	30	14	2	1.622789	0.208
Ovis aries	43	3	2	11.36288	**< 0.0001**
Rupicapra rupicapra	1	0	1	Inf	1
Sus scrofa	0	18	2	0	**< 0.0001**
Vulpes vulpes	0	4	1	0	0.054
Total	160	231	12[a]		

Notes: Fisher's exact test was used to determine whether the distribution of zoonotic and non-zoonotic strains varied significantly among host species. *P*-values are shown after the Benjamini-Hochberg procedure and in bold if significant.
[a] Number of unique countries.

higher McFadden's pseudo-R^2 (0.586) indicating that it explains a larger proportion of the variance and is a much stronger predictor of zoonotic potential. In contrast, the country model had a higher AIC (651.42) and a lower McFadden's pseudo-R^2 (0.228). Among the four tick species examined, *I. ricinus* was the sole species harbouring zoonotic haplotypes. Zoonotic haplotypes were detected in 6 of 311 *I. ricinus* specimens (1.9%, Table 2). Of the 1054 *A. phagocytophilum* Ecotype I isolates analysed, 128 (12%, Supplementary file 4: Fig. S2) carried a zoonotic haplotype, and all human isolates had an identical haplotype. The *groEL* sequences obtained from the five French and three Polish patients have been deposited in GenBank under accession numbers PV158090-PV158097.

4. Discussion

Ixodes ricinus is broadly distributed throughout Europe and so is *A. phagocytophilum* Ecotype I. Despite this overlap, HGA is only rarely diagnosed in Europe (Matei et al., 2019). This raises questions about the organism's pathogenic potential for humans. Other reasons for the low HGA case numbers in Europe might be mild illness, lacking awareness among health professionals or an insufficient sensitivity of current diagnostic protocols.

In the present study, we report (i) the first complete genome of *A. phagocytophilum* obtained from a European HGA patient and (ii) eight *groEL* sequences (366 bp) derived from isolates of HGA patients. The results were compared to molecular data from the literature. WGS, MLST, and *groEL* analyses revealed substantial genetic diversity among

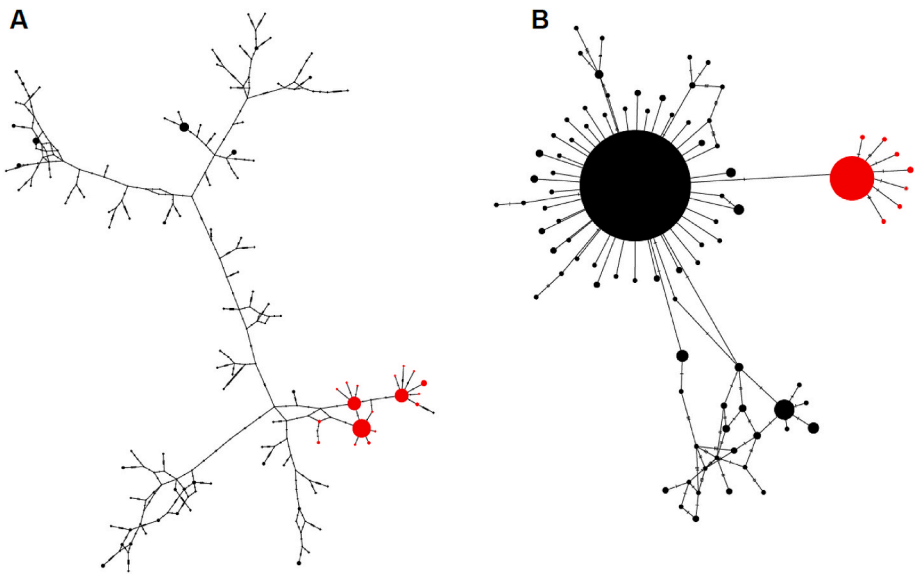

Fig. 2. **A** Median-joining haplotype network based on 391 concatenated housekeeping gene sequences (2877 bp) illustrating the relationships between different *A. phagocytophilum* variants within MLST Cluster 1. Each circle represents a unique ST. The circle size is proportional to the number of identical sequences. Clades containing sequences from human infections are highlighted in red. **B** Median-joining haplotype network based on 1054 sequences of the *groEL* gene (366 bp) belonging to Ecotype I. Each circle represents a unique haplotype. The circle size is proportional to the number of identical sequences. Clades containing sequences from human infections are highlighted in red. Hatch marks on the branches denote the number of nucleotide differences.

A. phagocytophilum strains from Europe as reported earlier (Aardema, 2023). The *A. phagocytophilum* genome from the Slovenian patient was more closely related to the *A. phagocytophilum* genomes from dogs ($n = 2$) than from sheep ($n = 2$). Due to the low number of available genome sequences, it was unclear whether the homology is associated with the host or the country of origin. MLST- and *groEL*-based typing indicated that only a subcluster of MLST Cluster 1 and Ecotype I contained human isolates, as it has been shown before (Huhn et al., 2014; Jaarsma et al., 2019; Langenwalder et al., 2020a).

These zoonotic clusters were defined to contain all human isolates. Isolates obtained from dogs, horses, cats, foxes, wolves, hedgehogs, and wild boars were statistically overrepresented in these clusters (Tables 1 and 2). The lineage harbouring the human strains was distinct from the other MLST Cluster 1 and Ecotype I variants circulating in wild and domestic ruminants (Supplementary file 3: Fig. S1, Supplementary file 4: Fig. S2) as has been shown before (Huhn et al., 2014; Jaarsma et al., 2019; Langenwalder et al., 2020a).

These results indicate that MLST Cluster 1 and Ecotype I are genetically heterogeneous and that only a subset of variants might be zoonotic. We propose two partially overlapping transmission cycles (Fig. 3). The zoonotic lineage probably circulates primarily between *I. ricinus* and horses, dogs, cats, carnivores, hedgehogs, and wild boar, but only occasionally spills over into humans. As granulocytic anaplasmosis in dogs and horses is common disease in Europe (Stuen et al., 2013), it remains to elucidate why this is a rare event.

On the other hand, the ruminant-associated lineage is maintained by *I. ricinus* and a broad assemblage of domestic and wild ruminants with no evidence of human infection. This partitioning summarized in Fig. 3, underscores the need for fine-scale genotyping in surveillance programs to differentiate truly zoonotic strains from those confined to animals. However, conversely, especially sheep were rarely found to be infected with zoonotic strains (Tables 1 and 2).

Although the MLST- and the *groEL* data were collated by convenience sampling, the zoonotic *A. phagocytophilum* variants were found in 17 European countries. Further, the eleven HGA cases with information on *groEl* typing originated from four countries spread across Europe. Based on the *groEL* data, the zoonotic variant was roughly 50 times less abundant in questing *I. ricinus* ticks than the non-zoonotic *A. phagocytophilum* Ecotype I variants (Table 2). For MLST Cluster 1, the zoonotic variant was roughly two times less abundant in *I. ricinus* ticks than the non-zoonotic variants. However, the comparison suffers from sampling bias because the number of *I. ricinus* ticks in the datasets (311 *vs* 44) and the number of countries (13 *vs* 2) was different. The occurrence of variants of *A. phagocytophilum* Ecotype I may vary locally depending on the host community. From the *groEL* analyses, we also infer that the zoonotic *A. phagocytophilum* variants are widespread in Europe, but far less abundant than the variants found in domestic and wild ruminants. These results help explain Europe's relatively low number of documented HGA cases. Thus, the actual risk is linked specifically to the density of questing *I. ricinus* ticks carrying the zoonotic MLST Cluster 1 and Ecotype I variant, which appears to be far less common than *I. ricinus* infection with *A. phagocytophilum* overall. Consequently, earlier estimates that treated all *A. phagocytophilum*-positive ticks as equally hazardous likely overstated the actual threat to humans. Another important finding is that the *A. phagocytophilum* variants responsible for disease in cattle and sheep are different from the variants causing granulocytic anaplasmosis in humans, as reported previously (Huhn et al., 2014; Langenwalder et al., 2020a).

Two major limitations of our study are the low number of *A. phagocytophilum* sequences from HGA patients and their comparison with sequence data collated from many different studies across Europe. This leads to a potential bias in the interpretation of the results. However, this limitation is inherent to eco-epidemiological studies of relatively rare infectious diseases, from which the etiological agents are difficult to detect, isolate, and cultivate (Azagi et al., 2020). In our dataset, strains from the European hedgehog *Erinaceus europaeus* were significantly overrepresented in the zoonotic branch for the MLST analyses (24/24), but this was not true for the *groEL*-based typing (0/5). From this, we infer that *E. europaeus* can act as a reservoir host, but it is unclear to what extent it can maintain the zoonotic variant in different environmental conditions. Clearly, more research is necessary to elucidate the host range and transmission dynamics of the zoonotic variant under different environmental conditions (e.g. rural *vs* forest areas).

We tried to address these limitations by performing three semi-independent analyses with high (WGS), medium (MLST) and low (*groEL*) genetic resolution on small ($n = 5$), medium ($n = 391$) and large ($n = 1054$) sample sizes, respectively. The finding that the zoonotic variants were distinct from the variants found in ruminants was

Table 2
European *A. phagocytophilum groEL* Ecotype I isolates were divided in a zoonotic and non-zoonotic branch which are inferred from Fig. 2. Only a subset of 989 out of 1054 isolates is shown. In total, 926 isolates were considered as non-zoonotic and 128 as zoonotic. For a complete list see Supplementary file 2. Species with fewer than 7 *A. phagocytophilum* isolates were omitted from this table for clarity.

Host species	Non-zoonotic	Zoonotic	Country	Odds ratio	*P*-value
Alces alces	27	0	3	Inf	0.215
Bison bonasus	15	0	2	Inf	0.492
Bos taurus	82	0	5	Inf	**< 0.001**
Canis familiaris	25	28	14	0.111659	**< 0.0001**
Canis lupus	4	7	2	0.077174	**< 0.001**
Capreolus capreolus	23	0	7	Inf	0.273
Cervus elaphus	127	0	10	Inf	**< 0.0001**
Dama dama	7	0	2	Inf	1
Equus caballus	11	16	5	0.089817	**< 0.0001**
Erinaceus roumanicus	18	0	3	Inf	0.329
Erinaceus sp	22	0	1	Inf	0.273
Felis catus	1	12	5	0.011024	**< 0.0001**
Homo sapiens	0	11	4	0	**< 0.0001**
Ixodes hexagonus	43	0	3	Inf	**0.026**
Ixodes ricinus	305	6	13	8.210424	**< 0.0001**
Ovis aries	112	5	6	3.230075	**0.021**
Ovis musimon	20	0	5	Inf	0.326
Rupicapra rupicapra	14	0	3	Inf	0.673
Sus scrofa	9	13	6	0.091469	**< 0.0001**
Vulpes vulpes	11	19	6	0.074709	**< 0.0001**
Total	872	117	24[a]		

Notes: Fisher's exact test was used to determine whether the distribution of zoonotic and non-zoonotic strains varied significantly among host species. *P*-values are shown after the Benjamini-Hochberg procedure and in bold if significant.
[a] Number of unique countries.

consistent and the most straightforward explanation. Furthermore, we accounted for the potentially spatial segregation present in the genetic information by collecting and analysing additional samples from HGA patients from Poland and France. Based on the distribution of the countries and hosts included in the phylogenetic analyses of the MLST and *groEL* sequences, we inferred that the genetic signal from the pathogen-host interaction was stronger than the pathogen-country interaction (Tables 1 and 2).

A further limitation is the possibility of mixed infections, in which a single host carries both the zoonotic and the non-zoonotic variants of *A. phagocytophilum* MLST Cluster 1 or Ecotype I. Because both variants can be detected in the same animal species (Tables 1 and 2) and *A. phagocytophilum* is probably capable of establishing persistent infections (Langenwalder et al., 2020b), standard diagnostic assays may fail to identify one or both variants in co-infected samples. This could lead to an underestimation of their true prevalence and host range. We previously developed a successful method to detect co-infections of *A. phagocytophilum* Ecotype I with Ecotype II (Gandy et al., 2022). Therefore, we think that the development of similar sensitive molecular tests that can detect co-infections with the zoonotic and non-zoonotic variants of *A. phagocytophilum* Ecotype I will be advantageous to further elucidate their enzootic cycles, geographical distributions and infection rates in ticks and hosts.

A molecular test for the specific detection of the zoonotic variants in questing ticks will enable us to more accurately assess the public health risk and possible preventive measures of HGA in different locations and situations in Europe.

Our findings indicate that there are at least two genetic variants of *A. phagocytophilum* MLST Cluster 1 or Ecotype I, which differ in their transmission dynamics between animals and ticks as well as in their ability to cause disease in humans. The phenotypic characteristics of these two variants are difficult to disentangle as they are both found in *I. ricinus*, share many vertebrate hosts and are widespread across Europe. The most characteristic difference is that the zoonotic variants have the ability to cause disease in humans, whereas the non-zoonotic variants do not. Whether the non-zoonotic variants might be able to cause asymptomatic infections in humans is not known. From the available data, the non-zoonotic variants have the ability to infect a broad range of animals, and the zoonotic genotypes only rarely infect ruminants (Fig. 3). From an ecological perspective, humans are considered accidental dead-end hosts. Thus, the genetic differences of the zoonotic variants might be driven by subtle differences in their transmission dynamics, which has been described for other *I. ricinus*-vectored pathogens as well (Genné et al., 2021; Bakker et al., 2024). Because we managed to obtain the complete genome of a zoonotic *A. phagocytophilum* isolate, genes that underlie those pathophysiological mechanisms can now be identified by comparison to the four other European *A. phagocytophilum* genomes and their effect can be analysed *in vivo*. This holds particularly true for horses and dogs as they can be infected with zoonotic and non-zoonotic variants of *A. phagocytophilum* Ecotype I (Table 2). It has already been shown that horses (Barlough et al., 1995; Madigan et al., 1995) and dogs (Scorpio et al., 2011) can be experimentally infected with North American *A. phagocytophilum* isolates of human origin. However, the respective strains did not undergo extensive genetic characterization.

There is currently no consensus on the nomenclature of different taxonomic units, not even for Cluster 1 (MLST) and Ecotype I (*groEL*). Such nomenclature is in place for *B. burgdorferi* (*sensu lato*) and *Babesia* species, which are subdivided into genospecies that differ in transmission dynamics and their ability to cause distinct disease manifestations (Coipan et al., 2016; Azagi et al., 2021). After the reclassification of the genus *Anaplasma*, three species previously classified as *Ehrlichia* (*E. equi*, *E. phagocytophila,* and the unnamed agent of human granulocytic ehrlichiosis) were unified to a single species named *A. phagocytophilum* (Dumler et al., 2001). With current knowledge, reconsidering a formally recognized subclassification of *A. phagocytophilum* may be advantageous for risk governance in both human and veterinary health. For this, acquiring genomic information of more *A. phagocytophilum* isolates from different sources is necessary to develop a robust nomenclature. Novel techniques are available to acquire whole-genome information from tick-borne pathogens without the necessity to isolate and cultivate the microorganism (Azagi et al., 2022). More insight into the genetics underlying the transmission dynamics and pathogenicity of the different variants of *A. phagocytophilum* MLST Cluster 1 or Ecotype I will help to improve preventive measures and diagnostic modalities for both human and veterinary health.

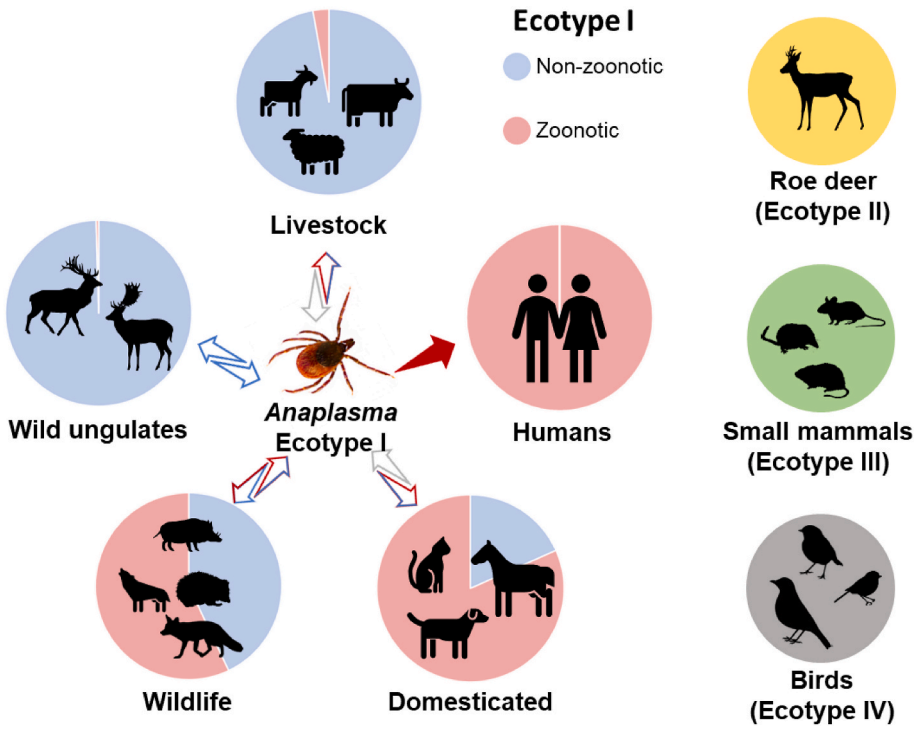

Fig. 3. Transmission cycle and host associations of *A. phagocytophilum* in Europe.
The figure depicts the proposed transmission cycle of *A. phagocytophilum* Ecotype I, highlighting two genetic variants: the zoonotic variant (in *red*) and non-zoonotic variants (in *blue*). Host species for Ecotypes I-IV are indicated. Ecotype I is associated with medium-sized to large mammals, with zoonotic variants rarely found in ruminants. Ecotypes II to IV show more restricted host ranges. Mixed infections in ticks and hosts are represented, illustrating potential challenges in detecting zoonotic variants in wildlife.

5. Conclusions

Human anaplasmosis in Europe is associated with a specific sub-cluster of *A. phagocytophilum* Ecotype I, which is not primarily associated with ruminants, but rather with dogs, horses, cats, carnivores, wild boar and hedgehogs. Our findings provide a reasonable explanation for the discrepancy between the omnipresence of *A. phagocytophilum* in the environment and the limited number of reported human cases. We recommend taking this genetic sub-clustering into account for future risk assessments.

Ethical approval

The clinical data of the patient have been described before as part of a cohort study (Lotric-Furlan et al., 2004). For this study, ethical clearance and informed consent from the patients were obtained for grant J3-4005 from the Ministry of Science and Technology of Slovenia.

CRediT authorship contribution statement

Paulina M. Lesiczka: Conceptualization, Methodology, Formal analysis, Investigation, Resources, Data curation, Writing - original draft, Writing - review & editing, Visualization, Funding acquisition. **Friederike D. von Loewenich:** Conceptualization, Investigation, Resources, Data curation, Writing - original draft, Writing - review & editing. **Robert Kohl:** Validation, Investigation, Writing - review & editing. **Aleksandra I. Krawczyk:** Methodology, Validation, Formal analysis, Writing - original draft, Writing - review & editing. **Ron P. Dirks:** Formal analysis, Investigation, Resources, Writing - original draft, Writing - review & editing, Supervision. **Pierre H. Boyer:** Investigation, Resources, Writing - review & editing. **Benoît Jaulhac:** Investigation, Resources, Writing - review & editing. **Anna Moniuszko-Malinowska:** Investigation, Resources, Writing - review & editing. **Tina Uršič:** Methodology, Writing - original draft, Writing - review & editing, Project administration. **Franc Strle:** Conceptualization, Methodology, Writing - review & editing, Supervision, Project administration, Funding acquisition. **Stanka Lotrič-Furlan:** Conceptualization, Writing - review & editing. **Tatjana Avšič-Županc:** Conceptualization, Methodology, Writing - review & editing, Supervision. **Miroslav Petrovec:** Conceptualization, Methodology, Formal analysis, Writing - original draft, Writing - review & editing, Supervision, Project administration, Funding acquisition. **Hein Sprong:** Conceptualization, Methodology, Resources, Writing - original draft, Writing - review & editing, Visualization, Supervision, Project administration, Funding acquisition.

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Declaration of competing interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.crpvbd.2025.100324.

Data availability

The genome sequence of *A. phagocytophilum* SLO-1 was submitted to GenBank under Bioproject number PRJNA1145303 and GenBank accession number CP166491. The Supplementary file 1 contains the description of the patient's history and the isolation and cultivation of *A. phagocytophilum* SLO-1. The *groEL* sequences from the other human isolates were deposited in GenBank under accession numbers PV158092-PV158097. Supplementary file 2 contains additional information on WGS, MLST, and *groEL*-based typing.

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