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Original article

Genetic characterization of *Anaplasma phagocytophilum* strains from goats (*Capra aegagrus hircus*) and water buffalo (*Bubalus bubalis*) by 16S rRNA gene, *ankA* gene and multilocus sequence typing

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ABSTRACT

Anaplasma phagocytophilum is a Gram-negative obligate intracellular bacterium that replicates in neutrophil granulocytes. It is transmitted by ticks and causes tick-borne fever in domestic ruminants such as sheep, cattle and goats. However, in contrast to sheep and cattle little is known about the clinical course of infection in goats. We report here on three cases of symptomatic infection with *A. phagocytophilum* in two goats (*Capra aegagrus hircus*) and one water buffalo (*Bubalus bubalis*). The animals showed symptoms and laboratory findings similar to sheep and cattle. To our knowledge, this is the first report on the symptomatic infection of water buffalos with *A. phagocytophilum.* The infecting strains were genetically characterized by 16S rRNA gene, *ankA* gene and multilocus sequence typing (MLST). Four other strains from asymptomatically infected goats were also included. The *ankA* sequences from five goats were part of the formerly described *ankA* gene clusters I and IV that are known to contain *A. phagocytophilum* strains from sheep and cattle. However, the sequences from one goat and from the water buffalo belonged to *ankA* gene cluster II that was formerly described to be restricted to roe deer. A similar observation was made for MLST as three goats clustered with sequences from sheep and cattle, whereas three other goats and the water buffalo were found to be part of the roe deer cluster. However, since most of the strains from sheep and cattle were distinct from the roe deer strains, roe deer might not represent major reservoir hosts for tick-borne fever in domestic ruminants. When differing parts of the 16S rRNA gene were used for typing the results were conflicting. This shows that the use of a standardized typing method such as MLST is highly desirable to generate easily comparable results.

1. Introduction

Anaplasma phagocytophilum is a Gram-negative obligate intracellular bacterium that replicates in neutrophil granulocytes forming bacterial inclusions in its host cells, so-called morulae (Dumler et al., 2001). It is generally transmitted by ticks of the *Ixodes persulcatus* complex. *I. ricinus* is its main vector in Europe (Stuen et al., 2013). *A. phagocytophilum* causes tick-borne fever in domestic ruminants such as sheep, cattle and goats (Atif, 2015). However, in contrast to sheep and cattle little is known about the clinical course of infection in goats. Other well-established mammalian species affected by *A. phagocytophilum* are dogs (Carrade et al., 2009), horses (Saleem et al., 2018), cats (Lappin, 2018) and humans (Ismail and McBride, 2017).

In general, clinical symptoms in domestic ruminants comprise fever,

Abbreviations: CC, clonal complex; ML, maximum-likelihood; MLST, multilocus sequence typing; NJ, neighbor-joining; ST, sequence type

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inappetence, cough, drop in milk yield and abortion (Stuen et al., 2013; Woldehiwet, 2010). Mainly in sheep, superinfections with *Listeria monocytogenes*, *Staphylococcus aureus* and *Mannheimia haemolytica* have been described (Woldehiwet, 2010). Goats experimentally infected with *A. phagocytophilum* showed similar laboratory changes as sheep and cattle and reacted with lymphopenia, neutropenia, thrombopenia and anemia (Gokce and Woldehiwet, 1999; van Miert et al., 1984v).

Clinically overt disease in goats with fever, anorexia and coughing has been reported from Scotland (Gray et al., 1988) and with cachexia, respiratory signs and abortion from north-eastern China (Zhan et al., 2010). Further, the presence of *A. phagocytophilum* in goats from Slovakia was associated with a prior history of frequent abortions in the herd (Čobádiová et al., 2013). In addition to the sparse reports on clinically ill goats, the description of asymptomatic infection with *A. phagocytophilum* exists from Europe (Silaghi et al., 2011). We report here on three cases of symptomatic infection with *A. phagocytophilum* in two goats (*Capra aegagrus hircus*) and one water buffalo (*Bubalus bubalis*). The infecting strains were genetically characterized by 16S rRNA gene, *ankA* gene and multilocus sequence typing (MLST). Four other strains from asymptomatically infected goats were also included to test whether certain genotypes are associated with subclinical infection.

2. Material and methods

2.1. Ethics statement

The samples were obtained as part of a routine diagnostic evaluation. Written informed consent was obtained from the owner.

2.2. Samples

Goat_1, goat_31298 and buffalo_740240 were symptomatically infected with *A. phagocytophilum* as it is described below. Goat 1 was already part of a former study (Huhn et al., 2014). Goat_A8July, goat_B10June, goat_B11June and goat_M8October showed no overt clinical signs and were reported earlier (Silaghi et al., 2011). Species and country of origin of the animals investigated here are shown in Table 1.

2.3. Sequencing

DNA from EDTA-anticoagulated blood samples was extracted using the High Pure PCR Template Preparation Kit (Roche, Mannheim, Germany). Two different parts of the 16S rRNA gene were partially amplified and bidirectionally sequenced as described earlier. Outer primers ge3a and ge10 r and inner primers ge9f and ge2 were used to generate a product of 497 bp (without primers) (Massung et al., 1998; von Loewenich et al., 2003v). The 497 bp-fragment was chosen, because it has been widely used before to characterize *A. phagocytophilum* strains. 599 bp (without primers) of the 16S rRNA gene were amplified with outer primers EE1 and EE2 (Barlough et al., 1996) and inner primers SSAP2f and SSAP2r (Kawahara et al., 2006). The 599-bp sequence was selected, because it was used to define *A. phagocytophilum* like-1 and *A. phagocytophilum* like-2 strains that were claimed to be non-pathogenic for domestic ruminants (Ben Said et al., 2015, 2017). The *ankA* gene was partially amplified and bidirectionally sequenced as described (Huhn et al., 2014). For MLST seven housekeeping genes (*pheS, glyA, fumC, mdh, sucA, dnaN, atpA*) were used as reported previously (Huhn et al., 2014). Clonal complexes (CC) were defined by sharing identical alleles at five of the seven loci with at least one other member of the group. The GenBank accession numbers of the sequences are shown in Table 2. The MLST profiles were submitted to the *A. phagocytophilum* isolates data base hosted at PubMLST (https://pubmlst.org/aphagocytophilum/).

Table 1
Species and country of origin of the animals.

Animal	Species	Country
goat_1	*Capra aegagrus hircus*	Germany
goat_31298	*Capra aegagrus hircus*	Switzerland
goat_A8July	*Capra aegagrus hircus*	Switzerland
goat_B10June	*Capra aegagrus hircus*	Switzerland
goat_B11June	*Capra aegagrus hircus*	Switzerland
goat_M8October	*Capra aegagrus hircus*	Switzerland
buffalo_740240	*Bubalus bubalis*	Switzerland

2.4. Phylogenetic analysis

The sequences described here were compared to *ankA* sequences from 392 samples (Huhn et al., 2014) and seven concatenated housekeeping gene regions from 314 samples without ambiguous nucleotides (Huhn et al., 2014; Tveten, 2014). The *ankA* and concatenated sequences were codon-aligned by ClustalW applying the PAM (Dayhoff) matrix. Trees were constructed using the neighbor-joining (NJ) method with the Jukes-Cantor model and the complete deletion option in the program MEGA X version 10.0.5 (Kumar et al., 2018). Bootstrap analysis was conducted with 1000 replicates. Maximum-likelihood (ML) analyses were also performed. For these, the best nucleotide substitution model for each dataset was determined in the program MEGA X with the small-sample-size corrected version of the Akaike Information Criterion (AICc). Using the most appropriate model the ML phylogenetic analysis were conducted with MEGA X generating 100 non-parametric bootstrap replicates to determine measures of nodal support with each run initiating from a random starting tree. NJ and ML phylogenies revealed comparable results (Figs. 1 and 2, Supplementary Figures S1 and S2).

3. Results

3.1. Case 1 (goat_1)

In 2011, the owner noticed inappetence, difficulties in breathing and neurological symptoms in a five-year old German fawn buck from the administrative district of Minden-Lübbecke in Germany. At the Clinic for Swine and Small Ruminants of the University of Veterinary Medicine Hannover the animal presented with fever of 40.1 °C (norm < 39.5 °C), lateral recumbency, trigeminal nerve paresis and tonic-clonic seizures. Blood and cerebrospinal fluid were drawn and the animal treated daily with 20 mg/kg oxytetracycline i.v., 2.2 mg/kg flunixin-meglumin i.v. and 245 µg/kg dexamethasone i.v over five days. Apart from a lymphopenia of 2.12 G/l (norm: 2.8–9.4 G/l) differential count and hemoglobin level were normal. Morulae of *A. phagocytophilum* were detected in a Giemsa-stained blood smear. In the cerebrospinal fluid a pleocytosis with leucocytes of 319 M/l was evident, but morulae were not found. Antibodies against *Listeria* antigen were detected in the cerebrospinal fluid. At the sixth day of illness the goat was euthanized. In the post-mortem analysis purulent bronchitis and lymphocytic meningoencephalitis were present. *L. monocytogenes* was isolated from the brain.

3.2. Case 2 (goat_31298)

In 2016, seven juvenile goats from a herd of 20 animals died in May at the beginning of the pasture season in the canton of Uri in Switzerland. The owner had noticed that the herd was highly tick-infested. Blood was drawn from one recumbent animal. The goat showed an anemia with erythrocytes of 4.0 T/l (norm 13.1–20.1 T/l), a hemoglobin level of 2.1 g/dl (norm: 7.4–12.3 g/dl) and a hematocrit of 7.4% (norm: 23.5–38.3%). The leukocytes were with 14.4 G/l (norm: 8.4–19.6 G/l) in the normal range, whereas the thrombocytes were elevated with 915.0 G/l (norm: 197.1–876.0 G/l). Further the aspartat-

Table 2
GenBank accession numbers of the 16S rRNA, *ankA* and the housekeeping gene sequences from the goats and the buffalo.

Animal	16S rRNA gene	*ankA*	*pheS*	*glyA*	*fumC*	*mdh*	*sucA*	*dnaN*	*atpA*
goat_1	KF242659 (497 bp) MK542837 (599 bp)	KF242660	KF245016	KF243971	KF243588	KF244354	KF245120	KF243205	KF242822
goat_31298	MK577313 (497 bp) MK542838 (599 bp)	MH997028	MH987484	MH987246	MH987127	MH987365	MH987603	MH987008	MH986889
goat_A8July	FJ538290(497 bp) MK542839 (599 bp)	MH987720	MH987485	MH987247	MH987128	MH987366	MH987604	MH987009	MH986890
goat_B10June	FJ538288 (497 bp) MK542840 (599 bp)	MH997029	MH987486	MH987248	MH987129	MH987367	MH987605	MH987010	MH986891
goat_B11June	FJ538289(497 bp) not done (599 bp)	MH987751	MH987487	MH987249	MH987130	MH987368	MH987606	MH987011	MH986892
goat_M8October	FJ538288* (497 bp) MK542841 (599 bp)	MH997030	MH987488	MH987250	MH987131	MH987369	MH987607	MH987012	MH986893
buffalo_740240	MK577314 (497 bp) MK542842 (599 bp)	MH987749	MH987435	MH987197	MH987078	MH987316	MH987554	MH986959	MH986840

* the sequence from goat_M8October was not submitted to GenBank, but is identical to FJ538288.

aminotransferase level was increased with 631 U/l (norm < 300 U/l). Morulae of *A. phagocytophilum* were present in the Giemsa-stained blood smear with an infection rate of 10% of the neutrophils. All animals were treated once with 8 mg/kg oxytetracycline i.v. and 0.2 mg/kg doramectine i.m. what let to rapid improvement.

3.3. Case 3 (buffalo_740240)

Since October 2013 several animals from a herd of water buffalos presented with fever, inappetence, recumbency, respiratory signs and drop in milk-yield. They went to pasture in the neighborhood of Lac Lucerne, Switzerland. The ill buffalos were empirically treated with 10 mg/kg oxytetracycline i.v. and showed rapid defervescence. In November 2013, blood was drawn from a female animal with high fever of 41.4 °C (norm < 39 °C) and inappetence. Morulae of *A. phagocytophilum* were present in the Giemsa-stained blood smear. The buffalo was treated once with 10 mg/kg oxytetracycline i.v. and 0.5 mg/kg meloxicam i.v. and made a rapid improvement.

3.4. 16S rRNA gene

Concerning the 497-bp fragment goat_1 and goat_31298 were infected with the same 16S rRNA gene variant that was identical to GenBank accession number M73220 (Table 3). M73220 was initially reported from sheep in Scotland (Anderson et al., 1991) and is often found in sheep (Huhn et al., 2014; Stuen et al., 2002) and cattle (Huhn et al., 2014; Nieder et al., 2012; Silaghi et al., 2018). As reported earlier goat_B10June and goat_M8October harbored a 16S rRNA gene variant identical to GenBank accession number AF136713 (Silaghi et al., 2011). The same was true for buffalo_740240. The respective variant was first detected in Swedish (von Stedingk et al., 1997v) and German (Baumgarten et al., 1999) *I. ricinus* ticks and is found primarily in roe deer and ticks (Huhn et al., 2014). The 497-bp sequence from goat_B11June was identical to GenBank accession number AF136714 which was initially described in a German *I. ricinus* tick (Baumgarten et al., 1999) and is mainly known from roe deer and ticks (Huhn et al., 2014). However, goat_A8July showed a unique sequence that was ascribed the GenBank accession number FJ538290 (Silaghi et al., 2011).

The 599-bp fragment could not be amplified from goat_B11June because the DNA was used up. All animals were infected with the same 16S rRNA gene variant that was identical to the *A. phagocytophilum* Hz strain (GenBank accession number CP000235) and has been found before in humans, dogs, horses, sheep and cattle.

3.5. ankA gene

Five *ankA* gene clusters I –V have been described so far (Majazki et al., 2013; Scharf et al., 2011). The partial *ankA* sequences analyzed here comprised 523 bp (cluster I), 529 bp (cluster II) and 535 bp (cluster IV), respectively. The *ankA* sequence from goat_A8July belonged to cluster I, the sequences from goat_B11June and buffalo_740240 to cluster II and the sequences from goat_1, goat_31298, goat_B10June and goat_M8October to cluster IV (Table 3, Fig. 1). The *ankA* sequences from goat_B10June and goat_M8October were identical to each other.

It has been shown earlier that the *ankA* cluster I contained among others *A. phagocytophilum* strains from humans, dogs, horses, sheep, cattle and red deer, whereas cluster IV harbored mainly those from sheep, cattle and red deer (Huhn et al., 2014). However, cluster II had been found to be restricted to roe deer and ticks what is in contrast to the data presented here.

3.6. Multilocus sequence typing (MLST)

Generally, different sequences of a given locus (*pheS, glyA, fumC, mdh, sucA, dnaN, atpA*) were ascribed a unique, but arbitrary allele number and each unique combination of alleles was assigned a sequence type (ST). The ST found in goat_1, goat_31298, goat_B10June, goat_B11June, goat_M8October and buffalo_740240 were unique and not described before (Table 3). In goat_A8July ST 55 was detected. ST 55 is part of clonal complex (CC) 5 that contains mainly *A. phagocytophilum* strains from humans, dogs and horses (Huhn et al., 2014). ST 220 found in buffalo_740240 formed together with two roe deer from an earlier study (Huhn et al., 2014) the new CC 13. The phylogenetic analysis of the concatenated allele sequences showed that goat_1, goat_31298 and goat_A8July were part of the formerly described MLST cluster I (Fig. 2) that contains mainly *A. phagocytophilum* strains from humans, dogs, horses, sheep and cattle (Huhn et al., 2014). However, the concatenated sequences of goat_B10June, goat_B11June, goat_M8October and buffalo_740240 were found to be placed in the MLST cluster II that has been described earlier to contain almost exclusively sequences from roe deer and ticks (Huhn et al., 2014).

4. Discussion

The two goats and the water buffalo presented here showed symptoms and laboratory findings typical for tick-borne fever. Except for goat_1 that suffered from superinfection with *L. monocytogenes* all animals promptly responded to oxytetracycline treatment which further supports the diagnosis of an *A. phagocytophilum* infection. Goat_31298 showed a marked anemia. In goats experimentally infected with *A. phagocytophilum* the anemia was only mild (Gokce and Woldehiwet, 1999; van Miert et al., 1984v). Thus, the severe anemia of goat_31298 could have been caused by superinfection with *A. ovis* or *Babesia* spp.

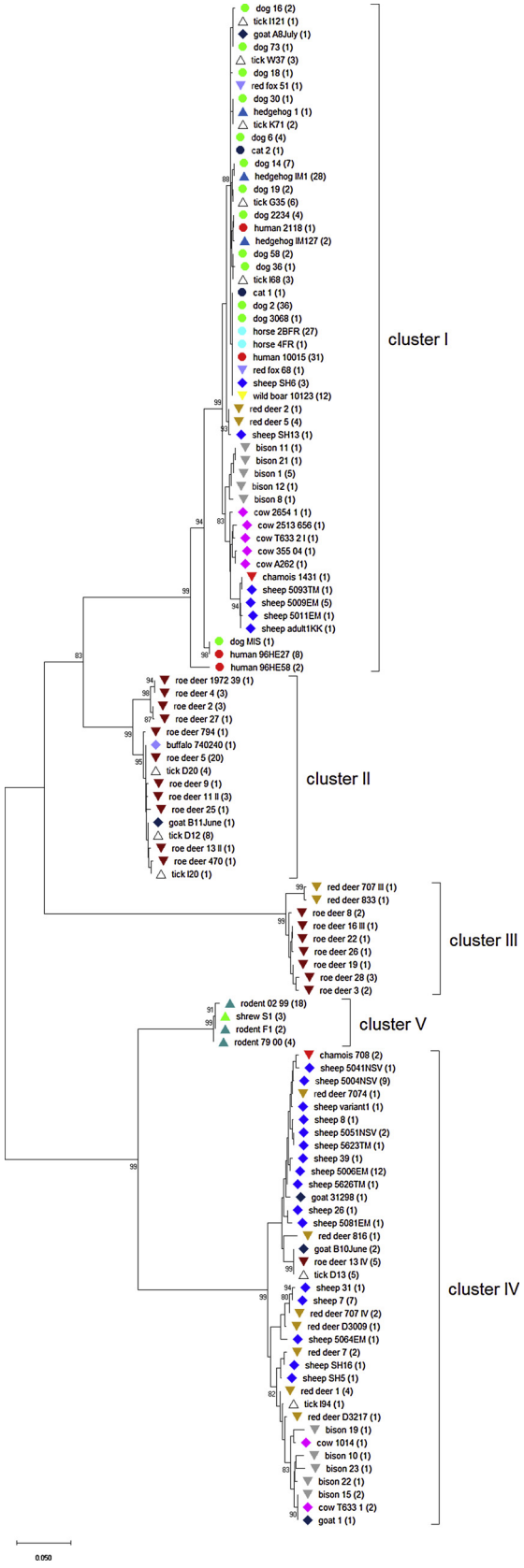

Fig. 1. NJ phylogenetic tree calculated from the *ankA* gene sequences.
Tree construction was achieved by the NJ method using the Jukes-Cantor matrix with the complete deletion option. Bootstrap values lower than 80% are not shown. The scale bar indicates the number of nucleotide substitutions per site. The final data set contained 500 positions. Identical sequences are displayed only once per species. The number in parenthesis indicates the frequency with which the respective sequence was found. Similar results were obtained using the ML method (Figure S1).
Symbols: human, dog, horse, cat, sheep, cattle, goat, water buffalo, roe deer, red deer, European bison, wild boar, chamois, red fox, hedgehog, vole, shrew, tick.

Specific PCR to detect such organisms was not done. However, intraerythrocytic inclusions were not observed in the blood smear.

In asymptomatic water buffalos, the infection with *Anaplasma* spp. that were not further differentiated has been detected earlier (Qiu et al., 2016). To our knowledge, we report here for the first time the symptomatic infection of water buffalos with *A. phagocytophilum*.

Several 16S rRNA gene variants of *A. phagocytophilum* have been described so far and it has been claimed that they differ in host adaptation and virulence (Rar and Golovljova, 2011). However, single locus 16S rRNA gene-based typing of *A. phagocytophilum* has been proven to not reliably define *A. phagocytophilum* genotypes (Bown et al., 2009, 2007; Casey et al., 2004; Huhn et al., 2014; Scharf et al., 2011; von Loewenich et al., 2003v). Further, the partial 16S rRNA gene sequences that have been looked at in the past differ considerably in position and length. This holds also true for *A. phagocytophilum* strains from goats. Sequences shorter than 400 bp were not considered here for discussion.

In China, *A. phagocytophilum* has been detected in goats with cachexia, respiratory signs and abortion (Zhan et al., 2010). The respective 16S rRNA gene sequence (GenBank accession number DQ342324) was 99.6% (1437/1442 bp) identical to the *A. phagocytophilum* Hz reference strain (GenBank accession number CP000235) what means that the infecting strain is correctly addressed as *A. phagocytophilum*. Further, an *A. phagocytophilum* strain that's 16S rRNA gene sequence was 99.3% (595/599 bp) identical to the *A. phagocytophilum* Hz type strain (GenBank accession number CP000235) was reported from asymptomatic goats in China (Yang et al., 2016). However, the amplified fragment was considerably shorter than that from the first study. In contrast, *Anaplasma* spp. reported as *A. phagocytophilum*, but only 98% identical to the *A. phagocytophilum* Hz type strain (GenBank accession number CP000235) were found in China in goats showing signs of depression, thin hair and weight loss (Yang et al., 2013) and in asymptomatic goats (Ge et al., 2016; Liu et al., 2012). The relevance of these strains for clinical illness in goats is unknown so far.

To further complicate the situation the 599 bp 16S rRNA gene fragment mentioned above was used to define *A. phagocytophilum* like-1 and *A. phagocytophilum* like-2 strains that were found in asymptomatic goats in Tunisia (Ben Said et al., 2015, 2017). *A. phagocytophilum* like-1 and *A. phagocytophilum* like-2 genotypes have been claimed to be non-pathogenic for domestic ruminants. For *A. phagocytophilum* like-1 strains a sequence from a deer from Japan (GenBank accession number JN055357) and for *A. phagocytophilum* like-2 strains a sequence from a *Hyalomma asiaticum* tick from China (GenBank accession number KJ410247) were used as type sequences (Ben Said et al., 2015, 2017). However, the 599 bp fragment is in our opinion too short to make a reliable ascription. Further, some of the *A. phagocytophilum* like-1 strains had a gap at position 1,057,862 when we aligned them to the *A. phagocytophilum* Hz strain (GenBank accession number CP000235), but others had not. Probably, this gap is a more stringent marker for strain difference than the single nucleotide exchanges.

In the study presented here, we obtained differing results depending on which 16S rRNA gene fragment was looked at. When 497 bp of a highly variable part of the 16S rRNA gene were used, all animals with exception of goat_A8July were infected with *A. phagocytophilum* variants previously known from sheep, cattle, roe deer and ticks (Huhn et al., 2014). However, if the 599 bp mentioned above were applied, the sequences of all animals were 100% identical to the *A. phagocytophilum* Hz reference strain (GenBank accession number CP000235) which was

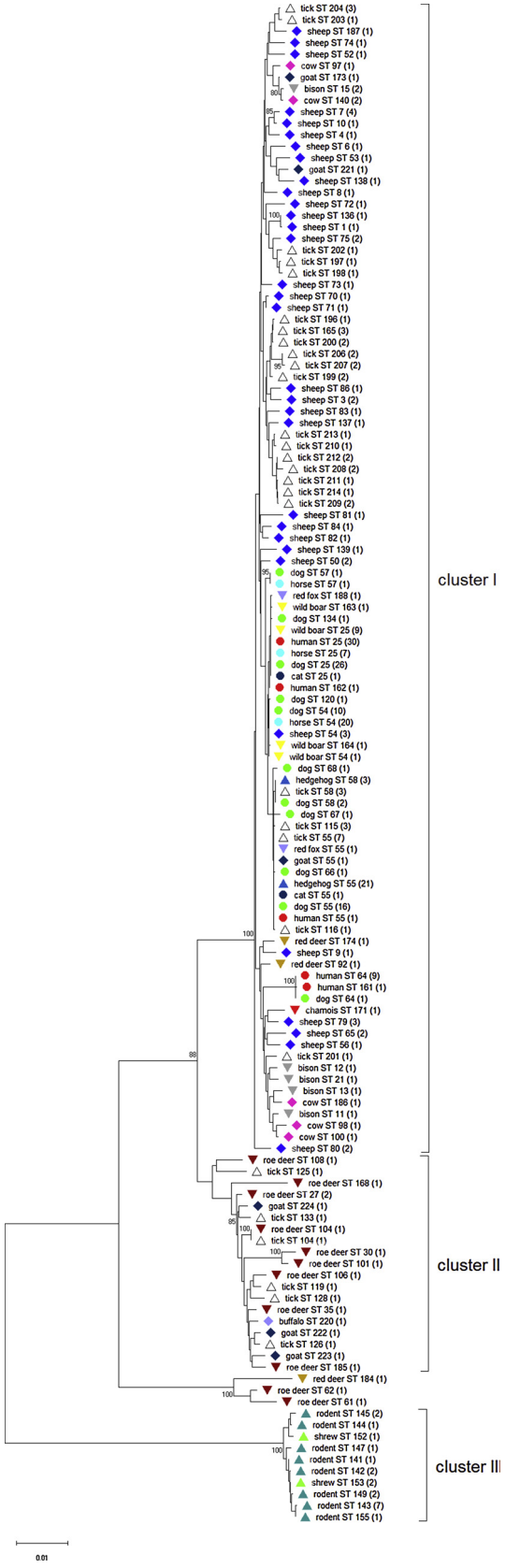

Fig. 2. NJ phylogenetic tree calculated from the concatenated housekeeping gene sequences.

Tree construction was achieved by the NJ method using the Jukes-Cantor matrix with the complete deletion option. Bootstrap values lower than 80% are not shown. The scale bar indicates the number of nucleotide substitutions per site. The final data set contained 2877 positions. Identical ST are displayed only once per species. The number in parenthesis indicates the frequency with which the respective ST was found. Similar results were obtained using the ML method (Figure S2).

Symbols: ● human, ● dog, ● horse, ● cat, ◆ sheep, ◆ cattle, ◆ goat, ◆ water buffalo, ▼ roe deer, ▼ red deer, ▼ European bison, ▼ wild boar, ▼ chamois, ▼ red fox, ▲ hedgehog, ▲ vole, ▲ shrew, ▽ tick.

isolated from a human (Rikihisa et al., 1997).

Because the 16S rRNA gene does not allow reliable strain identification as mentioned above, we also used the highly variable *ankA* gene for typing. As shown previously for sheep and cattle (Huhn et al., 2014), the *A. phagocytophilum* strains from five of the six goats were part of *ank*A gene clusters I or IV. However, the strains from goat_B11June and buffalo_740240 were found to be part of cluster II. *ankA* gene cluster II has been previously described to be restricted to roe deer (Huhn et al., 2014) what led to the hypothesis that roe deer might generally not serve as reservoir hosts for tick-borne fever in domestic ruminants. Using *groEL*-based typing an ecotype II has been defined that was overrepresented in roe deer, but underrepresented in sheep, whereas most of the *A. phagocytophilum* strains infecting sheep were part of ecotype I (Jahfari et al., 2014). A similar observation was made by a multilocus sequence approach that led to the description of three genetic clusters (Chastagner et al., 2014). The first cluster was restricted to cattle, the second one comprised strains from cattle, horses and dogs and the third one contained all genotypes from roe deer, but as well three genotypes from cattle. Thus, although *A. phagocytophilum* strains circulating in roe deer are apparently able to infect domestic ruminants, it seems that roe deer do not represent major reservoir hosts for tick-borne fever in domestic ruminants.

The MLST analysis revealed that goat_1, goat_31298 and goat_A8July were part of the formerly described MLST cluster I that contains mainly *A. phagocytophilum* strains from humans, dogs, horses, sheep and cattle (Huhn et al., 2014). However, the concatenated sequences of goat_B10June, goat_B11June, goat_M8October and buffalo_740240 were found to be placed in the MLST cluster II that has been described earlier to contain almost exclusively sequences from roe deer and ticks (Huhn et al., 2014). *A. phagocytophilum* strains from roe deer belonging to MLST cluster II, but to *ankA* gene cluster IV as those from goat_B10June and goat_M8October have been found before (Huhn et al., 2014). Thus, there seems to be no strict concordance between MLST and *ankA*-based typing. The three goats, goat_B10June, goat_B11June and goat_M8October were asymptomatically infected. Thus, it could be argued that *A. phagocytophilum* strains belonging to MLST cluster II were less pathogenic for domestic ruminants. However, buffalo_740240 infected with a MLST cluster II strain showed clinical illness.

In conclusion, we show here that the clinical picture of tick-borne fever in goats is similar to that in sheep and cattle. Further, we report for the first time the symptomatic infection of water buffalos with *A. phagocytophilum*. Third, when differing parts of the 16S rRNA gene of *A. phagocytophilum* were used for typing the results were conflicting. This shows that the use of a standardized typing method such as MLST is highly desirable to generate easily comparable results.

In the future, the analysis of whole genome sequencing data might replace conventional MLST. Whole genome sequencing data could be used to compare complete genomes or to infer core genome MLST. This would probably allow the construction of most reliable phylogenetic relationships. However, the isolation of *A. phagocytophilum* in cell culture from multiple hosts is cumbersome. Further, nucleic acid preparations from infected individuals contain huge amounts of host DNA compared to the content of *A. phagocytophilum* DNA what leads to the problem of a substantial quantity of background reads (Aardema ML and von Loewenich FD, unpublished results).

Table 3
Identity to GenBank accession number, *ankA* cluster, clonal complex (CC), sequence type (ST) and allele numbers in the animals analyzed.

Animal	16S rRNA gene variant (497 bp)	16S rRNA gene variant (599 bp)	*ankA* cluster	MLST cluster	CC	ST	*pheS*	*glyA*	*fumC*	*mdh*	*sucA*	*dnaN*	*atpA*
goat_1	M73220	CP000235	IV	I	13	173	17	2	3	3	11	65	28
goat_31298	M73220	CP000235	IV	I	4	221	106	80	3	4	5	79	28
goat_A8July	FJ538290	CP000235	I	I	NT	55	27	15	6	3	2	27	28
goat_B10June	AF136713	CP000235	IV	II	1	222	98	81	20	13	114	17	28
goat_B11June	AF136714	not done	II	II	NT	223	29	16	18	13	97	17	28
goat_M8October	AF136713	CP000235	IV	II	NT	224	2	84	18	13	99	13	28
buffalo_740240	AF136713	CP000235	II	II	NT	220	33	16	18	13	15	52	1

* NT = nontypeable.

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Declaration of Competing Interest

None.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.ttbdis.2019.101267.

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