Molecular detection of *Anaplasma phagocytophilum* DNA in Olive Baboons and Vervet monkeys in Kenya

Sophie Jerusa Masika (✉ masikasophie10@gmail.com)

University of Nairobi

Gerald Mwangi Muchemi

University of Nairobi

Joseph Muiruri Kamau

Institute of Primate Research

Tequiero Abuom

University of Nairobi

Samson Kamawe Mutura

Institute of Primate Research

Research Article

Keywords: Zoonosis, Olive baboons, Vervet monkeys, Kenya, *Anaplasma phagocytophilum*

Posted Date: April 8th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-355087/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Molecular detection of *Anaplasma phagocytophilum* DNA in Olive Baboons and Vervet monkeys in Kenya.

Sophie Jerusa Masika¹, Gerald Mwangi Muchemi¹, Joseph Muiruri Kamau², Tequero Abuom Okumu¹, Samson Kamawe Mutura²

Affiliations of authors

¹University of Nairobi, ²Institute of Primate Research

*Corresponding author. Tel., +254 724 855 023, E-mail address: masikasophie10@gmail.com

Abstract

Background

Nonhuman primates (NHPs) occupy an important place in zoonotic spill-overs, serving as either reservoirs or amplifiers of multiple neglected tropical diseases including tick-borne infections. Anaplasmosis is caused by obligate intracellular bacteria of the family *Anaplasmatatacae*. They are transmitted by Ixodid tick species and have a wide host range including wild animals, domestic animals and humans. The aim of this study was to establish the presence of *Anaplasma phagocytophilum* in Olive baboons and Vervet monkeys in Laikipia County, Kenya.

Results

A total of 164 whole blood samples, collected by USAID Predict II project from Laikipia County, Kenya, were included in the study. These comprised of 146 samples from Olive baboons (*Papio anubis*) and 18 from Vervet monkeys (*Chlorocebus pygerythrus*) respectively from Mpala Research Center and Ol jogi Conservancy in Laikipia County. Using conventional Polymerase Chain Reaction (PCR), positive results for *A. phagocytophilum* in 26 Olive baboons and 4 Vervet monkeys were detected with primer sets EHR16SD/R. However, low sensitivity was observed with the p44 gene. The amplification of DNA template with the primer set p44 (p3709 5' / p4257 5') using nested PCR could not be obtained. Our results revealed the presence of *A.*

phagocytophilum in Olive baboons and Vervet monkeys. This study found an overall prevalence of 18.3% for *Anaplasma phagocytophilum*. A distinct genotype of *A. phagocytophilum* was detected that was different from the others in the gene bank database.

Conclusion

This study provides valuable information on the presence of *A. phagocytophilum* bacteria in Olive baboons and Vervet monkeys in Kenya. It indicates a need for future research to establish the public health implications of zoonotic *A. phagocytophilum* isolates and the role of nonhuman primates as reservoirs.

Key words: Zoonosis, Olive baboons, Vervet monkeys, Kenya, *Anaplasma phagocytophilum*

Background

There has been a rise in the frequency of emerging infectious diseases (EIDs), among which zoonotic tick-borne infections especially rickettsial diseases such as anaplasmosis, are implicated [1]. What most of the recent pandemics have proven is that emerging infectious diseases are mainly of animal origin, particularly wildlife [2]. The complex interaction among wildlife, livestock and human populations is a factor that contributes to their emergence [3].

Among wildlife species, non-human primates are often proprietors to different microbial agents some which have zoonotic potential. Primates are closely related to humans phylogenetically and ecologically [4] and they can indirectly transmit infectious agents to humans through intermediate hosts, arthropod vectors or directly through bush meat consumption and accidental bites [5]. Certain factors such as forested tropical regions experiencing land-use changes and encroachment as well as those with a high wildlife biodiversity facilitate the spread of these diseases to livestock and man [6]. Others include adoption of new technology in farms, destruction of habitats, climate change, travel and encroachment into new habitats [2].

This study focused on *Anaplasma phagocytophilum*, a pathogenic bacterium of zoonotic potential often spread among wildlife then to livestock and man [7]. *Anaplasma* hemoparasites belong to the family of *Anaplasmataceae*, order of Rickettsiales, class Alphaproteobacteria and genus *Anaplasma* [8]. However, reclassification of *Anaplasma* happened recently resulting in *A. phagocytophilum* being included in the genus whose members were only pathogens with host specificity to ruminants such as *A. marginale*. This followed advancement in genetic analyses of 16S rRNA genes, groESL and surface protein genes. There is a considerable strain variation with *A. phagocytophilum* such that there appears to be existence of serological cross-reactivity [9], a

minor degree of variation in the nucleotide sequences of the 16S rRNA, groESL, gltA, ank, and msp2 genes [10] and a difference in the host infectivity [11].

This bacterium has a wide host range including domestic animals, wildlife and man [12]. In man the disease is known as human granulocytic anaplasmosis (HGA) [13]. The disease often presents with influenza-like symptoms in animal and human hosts which include fever, anorexia, diarrhea, leukopenia and thrombocytopenia [7]. Ixodid ticks are important in their maintenance as vectors [14]. The emergence of Anaplasmataceae as human pathogens has gained the attention of scientific community. A recent surveys have shown human infection with anaplasmosis including in Venezuela [15] and one in Morocco where dog owners were infected with *A. phagocytophilum* [16].

Recent reports on animal anaplasmosis are available from France, Massachusetts, Brazil, Zambia Ethiopia and Kenya in both domestic and wild animals [17-22]. While *Anaplasma* in NHPs has been reported in some countries, its importance in NHPs in Kenya is not yet known which has therefore led us to investigate the occurrence of this bacteria in NHPs in Laikipia, Kenya.

The investigation focused on blood DNA presence of *A. phagocytophilum* in Olive baboons (*Papio anubis*) and Vervet monkeys (*Chlorocebus pygerythrus*) in Laikipia County, Kenya. Laikipia is part of Kenya's rangelands mainly inhabited by trans-human pastoralists. It also has a large wildlife population including primate species. These animals are found close to human settlements. The objectives of this present study were (i) to assess the prevalence of *A. phagocytophilum* in Olive baboons and Vervet monkeys and (ii) to establish whether the bacteria is genetically diverse.

Methods

Study area, sample population and sample size

This study was part of the USAID Predict II project whose aim was to collect targeted information to support the interventions to mitigate spread of zoonotic viruses with pandemic potential. The focus was on highest risk locations and interfaces, where animals and people share changing landscapes. The study area, Laikipia County (Figure 1) located in the Rift Valley of Kenya with co-ordinates of 005°N 36040°E, was picked as one of the locations. There is a diverse range of wildlife in the area, including NHPs with 8 species of them found in the area.

Figure 1: Map of the sampling sites.

This study focused on two NHP species: Olive baboons (*Papio anubis*) and Vervet monkeys (*Chlorocebus pygerythrus*), since they are closely related to humans phylogenetically and are implicated in most of the listed emerging human pathogens. The sampling sites Mpala research Centre and Ol jogi Conservancy (Figure 1) were conveniently chosen as large numbers of these species inhabit these sites. All the whole blood samples from wild Olive baboons and Vervet monkeys that had been collected by USAID Predict II project from Laikipia County were included in this study constituting the study's sample size of 146 Olive baboon and 18 Vervet monkey. The animals were not owned.

DNA extraction

Whole blood samples stored in TRIzol reagent at -80°C were retrieved and allowed to thaw at room temperature. Extraction of genomic DNA from each of the whole blood sample was done using the DNeasy Blood & Tissue Kit (Qiagen, Valencia, California, USA) following manufacturer's instructions.

Nested PCR

First set of primers (p3709-p4257) were used in a nested PCR run for amplifying the p44 gene of *Anaplasma* species in Olive baboons and Vervet monkeys (Table 1). Using the second set of primers (p3761-p4183), 1 µl of the product from the first amplification was used in a 25 µl reaction mixture. Reactions were performed in a final volume containing 18.25 µl double distilled RNase/DNase free water, 2.5 µl PCR buffer, 0.75 µl 1.5 Mm MgCl₂, 0.5 µl of 10 mM DNTPs, 0.5 µl of 10 µM forward primer, 0.5 µl of 10 µM reverse primer, 0.1 µl of 2.5 U Taq DNA polymerase and 1.0 µl of the DNA template. Thermal cycling profiles were as previously described [23].

Conventional PCR

The primer set EHR16SD/R was used in a simple PCR to amplify 345 bp fragment of *Anaplasma* 16S rRNA gene on the same samples with thermo-cycling profile as previously described [24]. PCR products were electrophoresed on 1% agarose gel to check the size of amplified fragments by comparison with a DNA molecular weight marker (1 Kb Plus DNA Ladder, Promega, Madison, USA).

DNA sequencing and data analysis

Ten selected positive *Anaplasma* spp. PCR products obtained with primers EHR16SD/R were purified with Thermo Scientific GeneJET PCR Purification Kit #K0701, #K0702 Protocol

according to the manufacturer's instructions. Purified DNA fragments were sequenced using an ABI PRISM 377 Genetic Analyzer (Applied Biosystems, USA), using the same forward and reverse primers (Table 1) of each PCR assay. Sequence assembly for forward and reverse primers was done using DNA Sequence Assembler v4 (2013), Heracle BioSoft [25]. The sequences were matched to those deposited in the GenBank database using BLAST search (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>). Multiple alignment of the sequences was done using BioEdit Sequence Alignment Editor (Hall, T.A. 1999). Construction of phylogenetic tree was done using Muscle 3.8 using the neighbor-joining method and visualization of the trees with FigTree v1.4.4 [26].

All methods were carried out in accordance with relevant guidelines and regulations.

Results

Molecular survey of Anaplasma species

A total of 164 blood samples from Olive Baboons and 18, Vervet monkeys were screened for *A. phagocytophilum* through nested PCR employing the two sets of primers for p44 genes (Table 1). However, all these samples tested negative. A re-run and troubleshooting was done to confirm the results still turned out negative. With the same samples, a different assay and conventional PCR using primers targeting 16S rRNA gene (Table 1), it amplified a specific band of approximately 345bp. The overall prevalence for *anaplasmosis* was 18.3% (30/164) as estimated by EHR16SD/R PCR (Table 1) with 17.8% and 22.2% in Olive baboons and Vervet monkeys respectively.

Table 1: Primers used for detection and/or characterization of *Anaplasma* species in the present study

Assay	Primer	Sequence 5' to 3'	Target gene	Amplicon size(bp)	References
Nested PCR					
PCR 1	p3709	GCTAAGGAGTTAGCTTATGA	p44	400	[17]
	p4257	AGAAGATCATAACAAGCATTG			
PCR 2	p3761	CTGCTCTKGCCAARACCTC	p44	300	[17]
	p4183	CAATAGTYTTAGCTAGTAACC			
Conventional PCR	EHR16SD	GGTACCYACAGAAGAAGTCC	16S rRNA	345	[18]
	EHR16SR	TAGCACTCATCGTTTACAGC			

Molecular characterization of Anaplasma phagocytophilum. 16S rRNA genotypes

Nine of the 10 PCR products were successfully sequenced on both DNA strands and generated nucleotide sequences with primers EHR16SD/R targeting 345bp of the 16S rRNA gene of *Anaplasma* spp. Multiple alignment of *Anaplasma* nucleotide sequences of 4 *A. phagocytophilum* isolates revealed that all the sequences were conserved except one 15A which was the most divergent in comparison to the rest with a lot of accumulated mutations and numerous insertions. They all shared 99% to 100% nucleotide similarity except 15A at 81% (Table 2).

Table 2: BLASTn analysis results using 16S rRNA sequences of isolates from Olive baboons and Vervet monkeys.

Samples sequences	Animal species	Homologous sequences	E values	Identity (%)
15A	Olive Baboons	<i>A.phagocytophilum</i>	5	81.65
41A	Olive Baboons	<i>A.phagocytophilum</i>	4	99.39
39A	Olive Baboons	<i>A.phagocytophilum</i>	3	99.39
28A	Olive Baboons	<i>A.phagocytophilum</i>	4	100

160 The sequences of the *A. phagocytophilum* isolates from Kenya were identical to those from Japan,
 161 South Korea, France, China, South Africa and Denmark. The species isolates were from human,
 162 cattle, ticks, dogs and rodents. The accession numbers were JQ622148.1, MF351963.1,
 163 KU559922.1, MG519284.1, KX810088.1, MH122888.1, MK814411.1, MK814407.1,
 164 MF582329.1, MK814412.1, MK271308.1, MH122891.1, AY776165.1, KT986058.1,
 165 KU513793.1, and KR611718.1 (Figure 2). Phylogenetic analysis revealed that the isolates from
 166 Japan, South Korea, France, China, South Africa and Denmark belonged to clade I but have recent
 167 common ancestor with the Kenyan isolates clustered into one clade II except isolate 15A which
 168 appeared separately and represented an outgroup (Figure 3).

169 **Figure 2:** Multiple sequence alignment of 16S rRNA gene for *A. phagocytophilum* isolates.

```

170 Contig_15A CTTGCTCCAGCTACTTTCGCACCTCAGCGTTAGTACCGGCCATTATTGCCGGGCGTT CAGG-CCACGTAGGCC
Contig_28A .G...CAG...CG.GG.AAT..GGAG.G.GC.AGCGTT.TT.GGA...ATT.....AA...G..T.....
Contig_39A .G...CAG...CG.GG.AAT..GGAG.G.GC.AGCGTT.TT.GGA...ATT.....AA...G..T.....
Contig_41A .G...CAG...CG.GG.AAT..GGAG.G.GC.AGCGTT.TT.GGA...ATT.....AA...AG..T.....
JQ622148.1 .G...CAG...CG.GG.AAT..GGAG.G.GC.AGCGTT.TT.GGA...ATT.....AA...G..T.....
MF351963.1 .G...CAG...CG.GG.AAT..GGAG.G.GC.AGCGTT.TT.GGA...ATT.....AA...G..T.....
KU559922.1 .G...CAG...CG.GG.AAT..GGAG.G.GC.AGCGTT.TT.GGA...ATT.....AA...G..T.....
MG519284.1 .G...CAG...CG.GG.AAT..GGAG.G.GC.AGCGTT.TT.GGA...ATT.....AA...G..T.....
KX810088.1 .G...CAG...CG.GG.AAT..GGAG.G.GC.AGCGTT.TT.GGA...ATT.....AA...G..T.....
MH122888.1 .G...CAG...CG.GG.AAT..GGAG.G.GC.AGCGTT.TT.GGA...ATT.....AA...G..T.....
MK814411.1 .G...CAG...CG.GG.AAT..GGAG.G.GC.AGCGTT.TT.GGA...ATT.....AA...G..T.....
MK814407.1 .G...CAG...CG.GG.AAT..GGAG.G.GC.AGCGTT.TT.GGA...ATT.....AA...G..T.....
MF582329.1 .G...CAG...CG.GG.AAT..GGAG.G.GC.AGCGTT.TT.GGA...ATT.....AA...G..T.....
MK814412.1 .G...CAG...CG.GG.AAT..GGAG.G.GC.AGCGTT.TT.GGA...ATT.....AA...G..T.....
MK271308.1 .G...CAG...CG.GG.AAT..GGAG.G.GC.AGCGTT.TT.GGA...ATT.....AA...G..T.....
MH122891.1 .G...CAG...CG.GG.AAT..GGAG.G.GC.AGCGTT.TT.GGA...ATT.....AA...G..T.....
AY776165.1 .G...CAG...CG.GG.AAT..GGAG.G.GC.AGCGTT.TT.GGA...ATT.....AA...G..T.....
KT986058.1 .G...CAG...CG.GG.AAT..GGAG.G.GC.AGCGTT.TT.GGA...ATT.....AA...G..T.....
KU513793.1 .G...CAG...CG.GG.AAT..GGAG.G.GC.AGCGTT.TT.GGA...ATT.....AA...G..T.....
KR611718.1 .G...CAG...CG.GG.AAT..GGAG.G.GC.AGCGTT.TT.GGA...ATT.....AA...G..T.....

```

171 The conserved regions are represented by the dots (.) while the variable regions are indicated by the letters
 172 representing the nucleotide A, C, G and T

Figure 3: Phylogenetic tree of the 16S rRNA gene from *A.phagocytophilum* isolates.

Discussion

An epidemiological surveillance of pathogens in NHPs found at the human-animal interface is important in developing strategies on prevention and control of emerging and re-emerging zoonotic diseases.

Therefore, this study reports the detection of *A. phagocytophilum* DNA within the studied localities with an overall prevalence of 18.3%. *A. phagocytophilum*, an obligate intracellular bacterium, is the agent of human granulocytic anaplasmosis, formerly known as human granulocytic ehrlichiosis [13]. *A. phagocytophilum* has been previously reported to infect a wide range of animal hosts in various parts of the world [17-19], in Africa, [20, 27] while in Kenya [21, 22, 28, 29]. This study adds further evidence on the occurrence of *A. phagocytophilum* in Olive baboons and Vervet monkeys in Kenya.

In this study, a total of 164 samples were tested and an overall prevalence for anaplasmosis was 18.3% and 17.8% and 22.2% in Olive baboons and Vervet monkeys respectively which was similar to that reported in a survey on cattle in Ethiopia [27]. This is however inconsistent with reports by other authors. For example, in Zambia reported a 13% prevalence in baboons and Rhesus macaques [20]. In Kenya, a study on *Anaplasma* hemoparasites in wild animals revealed high seroprevalence [29] while another reported 35.5% sero-prevalence in cattle in Nairobi, Kenya [22]. Studies have shown that *A. phagocytophilum* can persist for the lifetime of animals due to its effect on the glucose metabolic pathways for maintenance of infection and multiplication [30]. This explains the high detection rates of Anaplasma infection in certain animal populations. Different host species, sample sizes, diagnostic technique, study areas demography and endemicity disease status in each study region could also explain it.

In this study, primers targeting p44 and 16S rRNA genes of *A. phagocytophilum* were identified from previous studies and used for detection of *A. phagocytophilum* [23, 24]. However, there was a significant difference in the performance of these two assays. The primers targeting p44 gene did not yield any positive bands on PCR. This is in agreement with several studies in cattle [27], wild birds and bats [31] and in ruminants and ticks [32]. This can be explained by the high variability of p44 gene, which includes intra-species variability, with consequent protein polymorphism and the generation of antigenic variations [33, 34]. The 16S rRNA gene yielded positive bands and therefore reinforced that it is a good marker for detection of *Anaplasma* as reported by other studies [20, 27, 35].

Further sequencing analysis revealed that the sequences of 16S rRNA were very conserved not only between African isolates but also between the other isolates of world-wide origin agreeably with previous studies [36]. The sequences of the *A. phagocytophilum* isolates from Kenya were

identical to those from Japan, South Korea, France, China, South Africa and Denmark. The species isolates were from human, cattle, ticks, dogs and rodents (Figure 3). This is consistent with a study on baboons in Zambia [20]. Multiple alignment of *Anaplasma* nucleotide sequences of the *A.phagocytophilum* isolates revealed that all the sequences of samples from baboons were conserved except for 15A. It was the most divergent in comparison to the rest with a lot of accumulated mutations and numerous insertions (Figure 2).

Extensive molecular studies have enabled understanding of the genetic profiles, level of genetic relatedness or variations among isolates across the different geographical regions and animal species. The isolates from Japan, South Korea, France, China, South Africa and Denmark belonged to clade I but have recent common ancestor with the Kenyan isolates clustered into one clade II except isolate 15A which appeared separately and represented a kind of outgroup (Figure 3). Previous studies have reported genetic relatedness between *A. phagocytophilum* infecting animals and humans [15, 35]. Similarly, in this study isolates from Olive baboons appear to be genetically related to those of human (MF582329.1) (Figure 3).

This, to the best of our knowledge, this is among the first molecular detection and estimate of the prevalence of *Anaplasma phagocytophilum* in Kenya's Olive baboons and Vervet monkeys. Non-human primates have been shown to host different pathogens, including several *Anaplasma* species [34, 20]. We assume that they could serve as a good indicator of bacteria circulation in ecosystem and explain the persistence of anaplasmosis in domestic animals despite consistence control.

Conclusions

This paper reports the presence of novel *Anaplasma phagocytophilum* in Olive baboons and Vervet monkeys in Laikipia County, Kenya. These findings open new concerns about the specificity of the molecular assays used to detect different *Anaplasma* species in and provide useful molecular

235 information to elucidate the evolutionary history of bacterial species related to *A.*
236 *phagocytophilum*. Further studies are needed to investigate the role of Olive baboons and Vervet
237 monkeys in the maintenance of *Anaplasma* organisms to establish the public health implications
238 of zoonotic *A. phagocytophilum* isolates.

239 **Abbreviations**

240 NHPs: Nonhuman primates

241 USAID: United States AID

242 PCR: Polymerase chain reaction

243 DNA: Deoxyribonucleic acid

244 RNA: Ribonucleic acid

245 rRNA: Ribosomal Ribonucleic acid

246 EIDs: Emerging infectious diseases

247 HGA: Human granulocytic anaplasmosis

248 OR: Odds ratio

249 CL: Confidence level

250 CI: Confidence interval

251 dNTPs: Deoxyribonucleotide triphosphate

252 BLAST: Basic Local Alignment Search Tool

253 Msp: Major Surface Protein

References

1. Heymann, D. L. *et al.* (2015). Global health security: the wider lessons from the West African Ebola virus disease epidemic. *Lancet* 385, 1884–1901
2. Jones B.A., Grace D., Kock R., Alonso S., Rushton J., and Said M.Y., McKeever D., Mutua F., Young J., McDermott J. & Pfeiffer D.U. (2013): Zoonosis emergence linked to agricultural intensification and environmental change. *Proc. natl Acad. Sci. USA*, 110: 8399–8404.
3. Brierley, L., Vonhof, M. J., Olival, K. J., Daszak, P. & Jones, K. E. (2016). Quantifying global drivers of zoonotic bat viruses: a process-based perspective. *Am. Nat* 187, E53–E64
4. Locatelli, S. & Peeters, M. (2012) Non-Human Primates, Retroviruses, and Zoonotic Infection Risks in the Human Population. *Nature Education Knowledge* 3(10):62
5. Jones-Engel L, May CC, Engel GA, Steinkraus KA, Schillaci MA, et al. (2008): Diverse contexts of zoonotic transmission of simian foamy viruses in Asia.
6. Allen, T., Murray, K.A., Zambrana-Torrel, C. et al. Global hotspots and correlates of emerging zoonotic diseases. *Nat Commun* 8, 1124 (2017). <https://doi.org/10.1038/s41467-017-00923-8>
7. Ismail N, Bloch KC and McBride JW (2010): Human ehrlichiosis and anaplasmosis. *Clinics in Laboratory Medicine* 30; 261–292.
8. Dumler, J. S., A. F. Barbet, C. P. J. Bekker, G. A. Dasch, G. H. Palmer, S. C. Ray, Y. Rikihisa, and F. R. Rurangirwa (2001): Reorganization of the genera in the families Rickettsiaceae and Anaplasmataceae in the order Rickettsiales: unification of some species of *Ehrlichia* with *Anaplasma*, *Cowdria* with *Ehrlichia* and *Ehrlichia* with *Neorickettsia*,

descriptions of six new species combination and designation of *Ehrlichia equi* and ‘HGE agent’

9. Wuritu, *et al.* (2009): Structural analysis of a p44/msp2 expression site of *Anaplasma phagocytophilum* in naturally infected ticks in Japan. *J. Med. Microbiol.* 58:1638–1644.
10. Lin Q, Rikihisa Y, Felek S, Wang X, Massung RF, Woldehiwet Z (2004): *Anaplasma phagocytophilum* has a functional msp2 gene that is distinct from p44. *Infection and Immunity* 72, 3883–3889.
11. Foley J. E., *et al.* (2009): Distinct ecologically relevant strains of *Anaplasma phagocytophilum* *Emerg. Infect. Dis.* 15:842–843
12. Kocan, Katherine & de la Fuente, Jose & Cabezas-Cruz, Alejandro (2015): The genus *Anaplasma*: New challenges after reclassification. *Revue scientifique et technique* (International Office of Epizootics). 34: 577-586.
13. Bakken JS, Dumler JS, Chen SM, Eckman MR, Van Etta LL, Walker DH. Human granulocytic ehrlichiosis in the upper Midwest United States. A new species emerging? *JAMA.* 1994 Jul 20; 272 (3):212-8. PMID: 8022040.
14. Zaid, T., Ereqat, S., Nasereddin, A., Al-Jawabreh, A., Abdelkader, A., & Abdeen, Z. (2019). Molecular characterization of *Anaplasma* and *Ehrlichia* in ixodid ticks and reservoir hosts from Palestine: a pilot survey. *Veterinary Medicine and Science.* doi:10.1002/vms3.150
15. Arraga-Alvarado CM, Qurollo BA, Parra OC, Berrueta MA, Hegarty BC, Breitschwerdt EB. Case report: Molecular evidence of *Anaplasma platys* infection in two women from Venezuela. *Am J Trop Med Hyg.* 2014 Dec; 91(6):1161-5. doi: 10.4269/ajtmh.14-0372. Epub 2014 Sep 29. PMID: 25266347; PMCID: PMC4257640.

16. Elhamiani Khatat, S., Daminet, S., Kachani, M. et al. (2017) *Anaplasma* spp. in dogs and owners in north-western Morocco. *Parasites Vectors* 10, 202 doi:10.1186/s13071-017-2148-y
17. Afonso E, Goydadin A-C (2018). Molecular detection of *Anaplasma phagocytophilum* DNA in the lesser horseshoe bat (*Rhinolophus hipposideros*) guano. *Epidemiology and Infection* 146,1253–1258.<https://doi.org/10.1017/S0950268818001279>
18. Galemone, E. R., Labato, M. A., & O’Neil, E. (2018): Prevalence of *Anaplasma phagocytophilum* infection in feral cats in Massachusetts. *Journal of Feline Medicine and Surgery Open Reports*,
19. Silveira, J. A. G., Rabelo, E. M. L., & Ribeiro, M. F. B. (2011). Molecular Detection of Tick-Borne Pathogens of the Family Anaplasmataceae in Brazilian Brown Brocket Deer (*Mazama gouazoubira*, Fischer, 1814) and Marsh Deer (*Blastocerus dichotomus*, Illiger, 1815). *Transboundary and Emerging Diseases*, 59(4), 353–360.
20. Nakayima J, Hayashida K, Nakao R, Ishii A, Ogawa H, Nakamura I, Moonga L, Hang’ombe BM, Mweene AS, Thomas Y, Orba Y, Sawa H, Sugimoto C (2014): Detection and characterization of zoonotic pathogens of free-ranging non-human primates from Zambia. *Parasit Vectors* 7:490
21. Kitaa, Jafred. (2014): A retrospective Study of Canine Ehrlichiosis in Kenya. *International Journal of Veterinary Science*. 3. 122-124
22. Peter SG, Gakuya DW, Maingi N, Mulei CM (2019) Prevalence and risk factors associated with *Ehrlichia* infections in smallholder dairy cattle in Nairobi City County, Kenya, *Veterinary World*, 12(10): 1599-1607.

23. Lin, Q., N. Zhi, N. Ohashi, H. W. Horowitz, M. E. Agüero-Rosenfeld, J. Ruffalli, G. P. Wormser, and Y. Rikihisa. 2002. Analysis of sequences and loci of p44 homologs expressed by *Anaplasma phagocytophila* in acutely infected patients. *J. Clin. Microbiol.* 40:2981–2988.
24. Parola, Philippe & Roux, Veronique & Camicas, J & Baradji, Issa & Brouqui, Philippe & Raoult, Didier. (2000). Detection of Ehrlichiae in African ticks by polymerase chain reaction. *Transactions of the Royal Society of Tropical Medicine and Hygiene.* 94. 707-8. 10.1016/S0035-9203(00)90243-8.
25. DNA Baser Database. <https://www.dnabaser.com/download/DNA-Baser-sequence-assembler/index.html>. Assessed 13 June 2020.
26. Molecular Evolution, Phylogenetics and Epidemiology. <http://tree.bio.ed.ac.uk/>. Assessed 20 June 2020.
27. Teshale, S., Geysen, D., Ameni, G., Dorny, P., & Berkvens, D. (2018). Survey of *Anaplasma phagocytophilum* and *Anaplasma* sp. “Omatjenne” infection in cattle in Africa with special reference to Ethiopia. *Parasites & Vectors*, 11(1).
28. Wesonga F.D., Kitala P.M., Gathuma J.M., Njenga M.J., Ngumi P.N. (2010): An assessment of tick-borne diseases constraints to livestock production in a smallholder livestock production system in Machakos district, Kenya. *Livest. Res. Rural Dev*; 22
29. Ngeranwa, J., Shompole, S., Venter, E., Wambugu, A., Crafford, J., & Penzhorn, B. (2008). Detection of *Anaplasma* antibodies in wildlife and domestic species in wildlife-livestock interface areas of Kenya by major surface protein 5 competitive inhibition enzyme-linked immunosorbent assay. *Onderstepoort Journal of Veterinary Research*, 75(3), 199-205. doi: <https://doi.org/10.4102/ojvr.v75i3.95>

30. Cabezas-Cruz, A., Espinosa, P. J., Obregón, D. A., Alberdi, P., & de la Fuente, J. (2017).
Ixodes scapularis Tick Cells Control Anaplasma phagocytophilum Infection by Increasing
the Synthesis of Phosphoenolpyruvate from Tyrosine. *Frontiers in Cellular and Infection
Microbiology*, 7. doi:10.3389/fcimb.2017.00375
31. dos Santos LGF, Ometto T, de Araújo J, Thomazelli LM, Borges LP (2013) Absence of
Anaplasmatidae DNA in Wild Birds and Bats from a flooded Area in the Brazilian
Northern Pantanal. *Air Water Borne Diseases* 2: 113. doi:10.4172/2167-7719.1000113
32. Yang, Jifei & Liu, Zhijie & Qingli, Niu & Liu, Junlong & Xie, Jingying & Chen, Qiuyu &
Chen, Ze & Guan, Guiquan & Liu, Guangyuan & Luo, Jianxun & Yin, Hong. (2016).
Evaluation of different nested PCRs for detection of *Anaplasma phagocytophilum* in
ruminants and ticks. *BMC Veterinary Research*. 12. 10.1186/s12917-016-0663-2.
33. Barbet AF, Lundgren AM, Alleman AR, Stuen S, Bjöersdorff A, Brown RN, Drazenovich
NL, Foley JE. Structure of the expression site reveals global diversity in MSP2 (P44)
variants in *Anaplasma phagocytophilum*. *Infect Immun*. 2006 Nov; 74 (11):6429-37. doi:
10.1128/IAI.00809-06. Epub 2006 Sep 11. PMID: 16966408; PMCID: PMC1695497.
34. Dumler, J. S. (2005): *Anaplasma* and *Ehrlichia* Infection. *Annals of the New York
Academy of Sciences* 106: 361-373
35. Elhamiani Khatat, S., Daminet, S., Kachani, M. et al. (2017) *Anaplasma* spp. in dogs and
owners in north-western Morocco. *Parasites Vectors* 10, 202 doi:10.1186/s13071-017-
2148-y
36. Zhang L, Wang G, Liu Q, Chen C, Li J, Long B, Yu H, Zhang Z, He J, Qu Z, YuJ, Liu Y,
Dong T, Yao N, Wang Y, Cheng X, Xu J (2013): Molecular analysis of *Anaplasma*

phagocytophilum isolated from patients with febrile diseases of unknown etiology in China. PLoSs One, 8:e57155–e57164.

Declarations

Ethical approval

Approval for this study was given by the Institute of Primate research Institutional Scientific and Ethics Review Committee (ISERC): Ethical approval number 1SERC/T01/18, 17 November. Since the information collected for this study was not of a sensitive nature and non-invasive as only blood samples previously collected were used, the ethical approval and guidelines received from this committee was deemed sufficient to conduct the study.

Consent for publication

Not applicable.

Availability of data and materials

Data and materials are available upon reasonable request from the corresponding author.

Competing interests

The authors declare that they have no competing interests.

Funding

The work was supported by the funding from the USAID Predict II project, 2018.

Authors' Contributions

SJM conducted the field and laboratory work. In addition, she participated in the study design, did the data analysis and preparation of this manuscript. GMM, JMK, and TAO were involved in the conception of the study, study design and the writing of this manuscript. SKM participated in the laboratory work. All authors have read and approved the final manuscript.

Acknowledgement

We thank the staff at the Molecular Laboratory at the Institute of Primate Research, Kenya who assisted with laboratory analysis of the work. The authors would like to thank Dr. Mercy Akinyi, and Dr. Lilian Waiboci for their invaluable contribution in the work.

Authors' information

Affiliations

1. Department of Public Health, Pharmacology and Toxicology, Faculty of Veterinary Medicine, University of Nairobi, Nairobi, Kenya

Sophie Jerusa Masika

2. Department of Public Health, Pharmacology and Toxicology, Faculty of Veterinary Medicine, University of Nairobi, Nairobi, Kenya

Gerald Mwangi Muchemi

3. Reproductive Health and Biology Department, Institute of Primate Research, National Museums of Kenya, Nairobi Kenya.

Joseph Muiruri Kamau

404 4. Department of Clinical Studies, Faculty of Veterinary Medicine, University of Nairobi,
405 Nairobi, Kenya

406 Tequiero Abuom Okumu

407 5. Reproductive Health and Biology Department, Institute of Primate Research, National
408 Museums of Kenya, Nairobi Kenya.

409 Samson Kamawe Mutura, Joseph Muiruri Kamau

410 **Corresponding author**

411 Correspondence to Sophie Jerusa Masika, Tel., +254724855023, Email:

412 masikasophie10@gmail.com

413

Figures

Figure 1

Map of the sampling sites. Note: The designations employed and the presentation of the material on this map do not imply the expression of any opinion whatsoever on the part of Research Square concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. This map has been provided by the authors.

[illegible]

Figure 2

Multiple sequence alignment of 16S rRNA gene for *A. phagocytophilum* isolates. The conserved regions are represented by the dots (.) while the variable regions are indicated by the letters representing the nucleotide A, C, G and T

Figure 3

Phylogenetic tree of the 16S rRNA gene from *A. phagocytophilum* isolates.