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Original Article

A glimpse of the bacteriome of *Hyalomma dromedarii* ticks infesting camels reveals human *Helicobacter pylori* pathogen

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Abstract

Introduction: The tick *Hyalomma dromedarii* is predominant in camels of Saudi Arabia and harbor multiple pathogens causing disease in humans and animals. Knowing the bacterial community of ticks is crucial for surveillance of known and newly emerging pathogens. Yet, the bacteriome of *H. dromedarii* remain unexplored to date.

Methodology: In a cross-sectional survey, we used V3-V4 region of 16S rRNA to characterize the bacteriome of 62 whole *H. dromedarii* tick samples collected from camels found in Hofuf city in Saudi Arabia.

Results: Sequencing results yielded 217 species incorporated into 114 genera, which in turn belong to the dominant phylum *Proteobacteria* (98%) followed by *Firmicutes* (1.38%), *Actinobacteria* (0.36%), *Bacteroidetes* (0.1%), meanwhile the phyla *Cyanobacteria*, *Verrucomicrobia* and unclassified bacteria were rarely detected. *Francisella* endosymbiont dominated the bacteriome of *H. dromedarii* ticks with average abundance of 94.37% and together with *Falincocc us* sp. accounted for 94.51% of the average sequences. The remaining bacteriome consisted of low abundance of potential pathogens and environmental bacteria. Of these pathogens, we found *Helicobacter pylori* in the tick *H. dromedarii* for the first time. Notably, *Anaplasma*, *Ehrlichia* and *Rickettsia* pathogens known to be found in *H. dromedarii* ticks were not detected.

Conclusion: This first preliminary study advances our knowledge about the bacterial community of *H. dromedarii* ticks and provides a basis for pathogen surveillance and studying the influences of symbionts on vector competence. Presence of pathogens in ticks, raise concerns about potential transmission of these agents to humans or animals.

Key words: Tick; *Hyalomma dromedarii;* bacteriome.

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Introduction

Hyalomma dromedarii is a species of hard-bodied ticks in the family *Ixodidae* that parasitizes several domestic ungulate animals [1] and the most commonly reported tick attached to camels in Saudi Arabia [2]. The tick *H. dromedarii* harbors several human and animal pathogens such as Crimean-Congo hemorrhagic fever virus [3], Alkhurma hemorrhagic fever virus [4], *Theileria camelensis* [5] and *Rickettsia* species [5]. Thus, *H. dromedarii* ticks are suspected to play a role in the epidemiology of these pathogens. Notably, most prior studies of *H. dromedarii* in Saudi Arabia have focused on viral and protozoan pathogens but not on bacterial agents [4-5]. Moreover, globally studies have screened *H. dromedarii*-borne bacteria but using species-specific PCR-based assay. Consequently, a complete screen of the *H. dromedarii* bacteriome is needed. Currently, the 16S rRNA metataxonomics analyses circumvent the limitation of previous methods, facilitating detection of more bacterial communities in ticks. From the epidemiological point of view, comprehensive analysis of bacteria residing in ticks of veterinary and medical importance is decisive for monitoring and surveillance of diseases and newly emerging zoonotic pathogens circulating in ticks. So far, the tick microbiome of the genus *Hyalomma* has only been characterized in the species *H. rufipes, H. annotilucm, H. isaaci, H. scupense, H. aegyptium*, *H. marginatum and H. excavatum* [7-9] by 16S rRNA metataxonomic approach. Since previous studies already determined that the tick microbiome can alter with a number of factors such as ticks feeding, environment and life stage [10,11], the analysis of these factors is beyond the scope of this study. Therefore, the aim of this study was to survey pathogens of *H.*

dromedarii ticks in the eastern province of Saudi Arabia using 16S rRNA metataxonomic approach.

Methodology

Tick collection and DNA extraction

In a cross-sectional study, a total of 62 adult *H. dromedarii* tick isolates were collected from camels at the local animal trade market in Al Hofuf, eastern province, where camels are brought to the market daily from different sites in Saudi Arabia. Samples were collected from April to May in 2017. Ticks were collected from camels and placed in sterile Falcon 50mL conical centrifuge tubes. Collected ticks were stored at −20 °C, before whole tick DNA was extracted using the DNeasy Blood &Tissue extraction kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions and kept at −20 °C until used as a template for PCR amplification.

Tick identification

Morphological and molecular identification were used for tick identification. PCR amplifications of ribosomal 16S rRNA were generated with published primers [12]. Two microliters of tick DNA and 0.3 µL of each primer (10 pmol) (Macrogen, Seoul, South Korea) were added to the PCR mixture, containing one unit of Max Taq DNA Polymerase (Vivantis Technologies, Subang Jaya, Malaysia), 5 µL of 10X ViBuffer (Vivantis Technologies, Subang Jaya, Malaysia) and 2 µL of dNTPs (10 mM). The volume was adjusted to 25 µL by adding distilled water. Thermal cycling was performed on a Tpersonal Thermocycler (BIOMETRA, Gottingen, Germany) with an initial 15-minutes cycle at 95°C followed by 35 cycles consisting of 30 seconds at 94°C, 1 minute at 55°C or 60°C depending on the primer and 1 minute at 72°C, followed by a 10 minutes final extension step at 72°C. To rule out DNA or amplicon contamination, molecular grade water negative control was used throughout the steps of the protocol. The PCR amplicons were sequenced by Macrogen Inc. (Seoul, South Korea) using BigDye (Applied Biosystems Foster city, CA, USA) on ABI3730XL DNA sequencer (Applied Biosystems Foster city, CA, USA)

Francisella sp. classification

The identification of *Francisella* sp. to the species level was performed via PCR of total DNA of 3 randomly selected individual ticks, using 16S rRNA *Francisella*-specific primers Fr153F0.1 (5-GCCCATTTGAGGGGGATACC-3) and Fr1281R0.1 (5-GGACTAAGAGTACCTTTTTGAGT-3) as mentioned before [13]. Sequencing was performed by Macrogen Inc. (Seoul, South Korea) using BigDye (Applied Biosystems, Foster city, CA, USA) on ABI3730XL DNA sequencer (Applied Biosystems Foster city,CA, USA). The obtained sequences were blasted against NCBI non-redundant (nr) database to find the closest species.

V3-V4 16S rRNA amplicon sequencing and Bioinformatics analysis

Prior to 16S rRNA library preparation, extracted DNA samples were pooled because of the high financial burden of performing sequencing for each individual tick. The DNA of 62 individual ticks were divided into two individual samples (consisting of sample 99 and 100) and eight pooled samples (named 28, 29, 30, 55, 64, 65, 71 and 73.01). Each pool composed of 7 tick DNA samples (total 42 DNA samples) except pool 71 and 73.01 each composed of 9 tick DNA samples (total 18 DNA samples). Ticks were grouped based on sex and engorged or non-engorged ticks. Each pool composed of male and female plus engorged and non-engorged ticks. The number of males, female, engorged and non-engorged ticks between pools was not the same due to their unequal number in the collected samples. For instance, the 62 adult ticks consisted of 27 engorged and 35 non-engorged ticks. Therefore, we have unequally number of engorged and non-engorged ticks. This distribution will not affect our main goal which is to screen pathogen and not to estimate difference between female and male ticks or engorged and non-engorged ticks which is published previously in other tick species.

Briefly, The V3-V4 segment of the 16S rRNA gene was amplified with Bakt_341F and Bakt_805R primers [14]. The amplicon library was constructed by ligating sequencing adapters and indices to purified PCR products using the Nextera XT Kit (Illumina, San Diego, CA, USA) according to the 16S rRNA metataxonomics sequencing library preparation protocol (Illumina, San Diego, CA, USA). Then libraries concentration was measured, equimolar volume of each of the libraries was pooled and sequenced on an Illumina's Miseq platform with paired-end 300 bp reads by Macrogen Inc (Seoul, South Korea). MiSeq reads were assembled by FLASH version 1.2.11 [15] which merge overlapping paired-end reads. Read trimming, filtering with a quality score offset 33 and OTU picking with a 97% identity cut-off was performed using CD-HIT-OTU software [16]. OTUs were classified by blast against NCBI 16S rRNA database with BLASTN using default parameters [17].

QIIME software was used to assign taxonomy and perform rarefaction curves and alpha diversity analyses (Chao1 index and sample coverage) [18].

For species-level identification using V3-V4 16S rRNA sequences region, Villmones *et al*. 2018 [19] recommends > 99.3% similarity with a trusted reference species together with a minimum distance of > 0.8% to the closest species. Based on the levels of intra-species sequence variation we observed in Genbank sequences, we adopted a more stringent cut off ≥ 1 % minimum distance to the closest species while keeping a similarity of ≥ 99.3%.

Results

Taxonomic analysis of 16S rRNA sequencing data

All tick samples collected in this study were genetically identified by sequencing of partial 16S rRNA gene as *H. dromedarii* ticks. For 62 *H. dromedarii* tick DNA samples (2 individual, 8 pooled), we obtained after removal of low quality and chimeric reads, a total of 755,940 high quality reads. The observed rarefaction curves and chao1 rarefaction curves reached plateau for all samples (Figure 1). The Good's coverage estimates range between 0.99% to 1.00%. These results show that the sequencing depth was sufficient to estimate 99% of the bacterial diversity and species richness in all samples (Figure 1).

A total of 546 OTUs were identified at 97% sequence similarity level, which were assigned to 6 phyla, 70 families and 114 genera. The unclassified OTUs at the phylum and the genus level were 12 and 29 OTUs respectively (Table 1). The bacterial calculated richness varied from (51 to 57 OTUs) per individual samples and (26 to 106 OTUs) per pooled samples (Table 1). The lowest number of observed OTUs was 26 in sample 55, whereas the highest was 106 in sample 30 (Table 1).

At the phylum level, *Proteobacteria* was found to be the most dominant with average abundance of 98.12% followed by *Firmicutes* (1.34%), *Actinobacteria* (0.33%), *Bacteroidetes* (0.16%), meanwhile the phyla cyanobacteria, *Verrucomicrobia* and unclassified were rarely detected (Table 2). Among these phyla, *Proteobacteria, Firmicutes* and *Actinobacteria* were found to be present in all samples. The total number of bacteria assigned to *Firmicutes* was 214 OTUs followed by *Proteobacteria* (160 OTUs) and *Actinobacteria* (99 OTUs) other phyla contain less than 50 OTUs were shown in (Table 1).

Figure 1. (A) Rarefaction curves demonstrating the Chao1 index of pooled samples (B) Rarefaction curves demonstrating the observed species index of pooled samples. (C) Rarefaction curves demonstrating the Chao1 index of individual samples. (D) Rarefaction curves demonstrating the observed species index of individual samples.

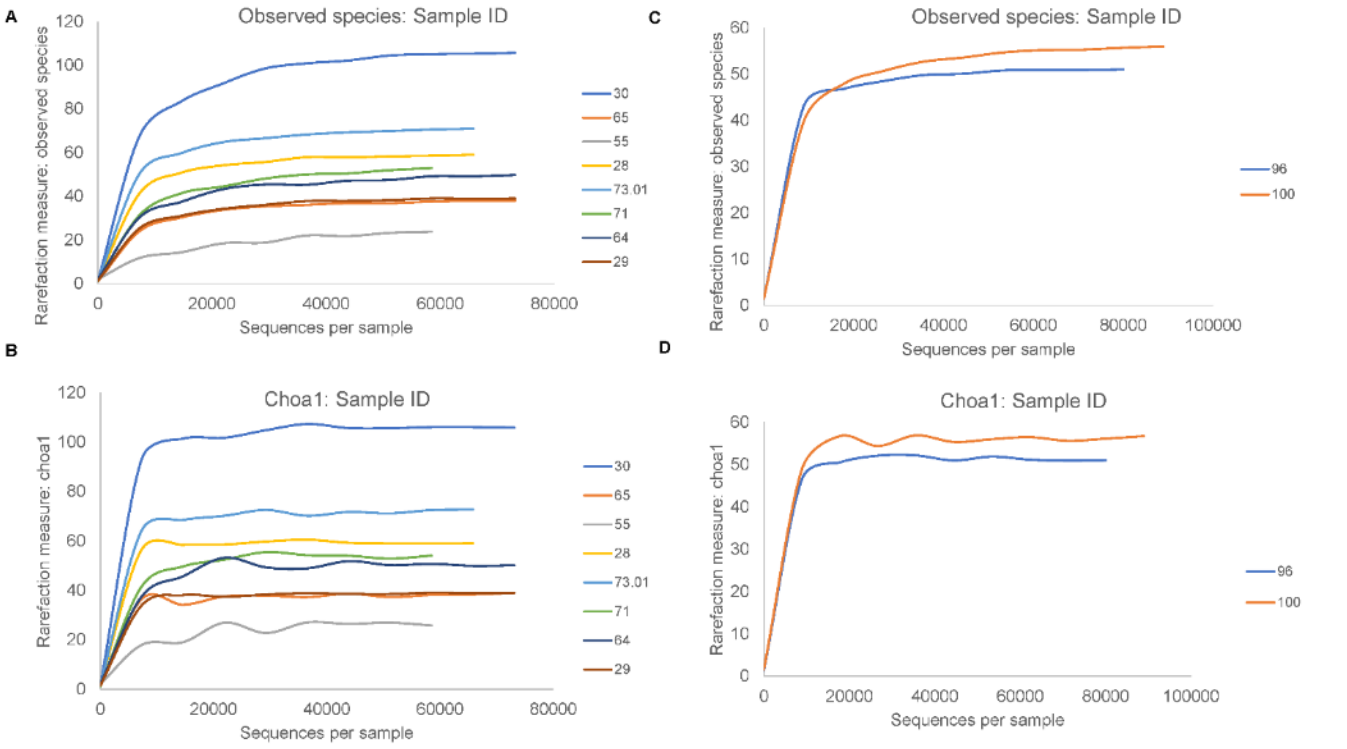


Figure 2. Neighbor-Joining rooted phylogeny of the V3-V4 16S rRNA sequences of *Helicobacter* species and OTU identified as *Helicobacter pylori* (Green circle) in this study. The bootstrap consensus tree inferred from 1000 replicates. Bootstrap values > 50% are shown. The evolutionary distances were computed using the Tamura-Nei method analyses were conducted in MEGA7 [58].

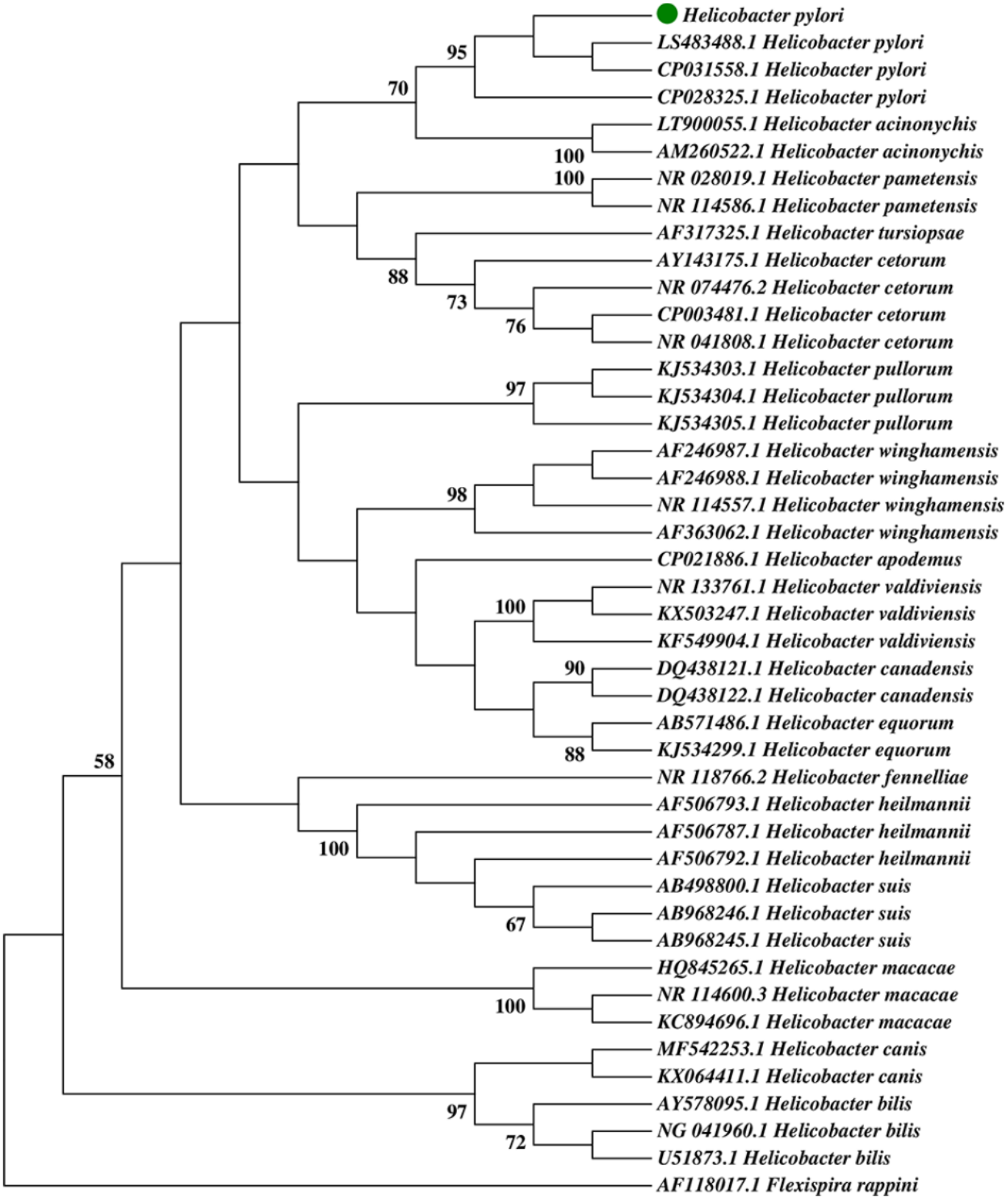


Table 1. Number of tick library analyzed, estimated OTU richness (Chao1), estimated sample coverage for 16S rRNA libraries and phylum level composition of OTUs.

TLN	Chao1	Goods coverage	Firm	Pro	Actin	Bact	Cyan	Verr	Unc	Total OTUs
			Number of OTUs							
28	59	0.999986145	23	22	9	4	1			59
29	39	1	13	13	5	4	1		3	39
30	106.333	0.999974448	52	21	16	11	1	2	3	106
55	26	0.999916963	10	10	3			1		24
64	50.5	0.999975745	18	19	11	1	1			50
65	39	0.999973005	19	9	7	1	1			37
71	53.5	0.999953493	20	20	9	1	1		2	53
73.01	72.5	0.999955544	25	12	20	5	1	1	7	71
96	51	1	13	18	7	11			2	51
100	57	0.999978875	21	16	12	7				56
Total			**214**	**180**	**95**	**48**	**7**	**4**	**17**	**596**

TLN: Tick library name; Firm: Firmicutes; Pro: Proteobacteria; Actin: Actinetobacteria; Bact: Bacterioidetes; Cyan: Cyanobacteria; Verr: Verrucomicrobia; Unc: Unclassified.

At the genus level, *Francisella* belonging to *Proteobacteria* phylum was the most abundant with average abundance of 94,37% (70,03% to 99,09) followed by *Proteus* (*Proteobacteria*) 2,97% (0,03-29,70), *Staphylococcus* (*Firmicutes*) 0,51% (0,05-2,22), *Acinetobacter* (*Proteobacteria*) 0,46% (0,02-3,83), *Corynebacterium* (*Actinobacteria*) 0,25% (0,01-0,89), *Salinicoccus* (*Firmicutes*) 0,21% (0,03-0,48), *Pseudomonas* (*Proteobacteria*) 0,14% (0,01-0,53), *Enterococcus* (*Firmicutes*) 0,12% (0,05-0,36) and *Solibacillus* (*Firmicutes*) 0,1% (0,04-0,48) (Supplementary Table 1). Other genera having abundance less than 0,1% were listed in (Supplementary Tables 2 and 3).

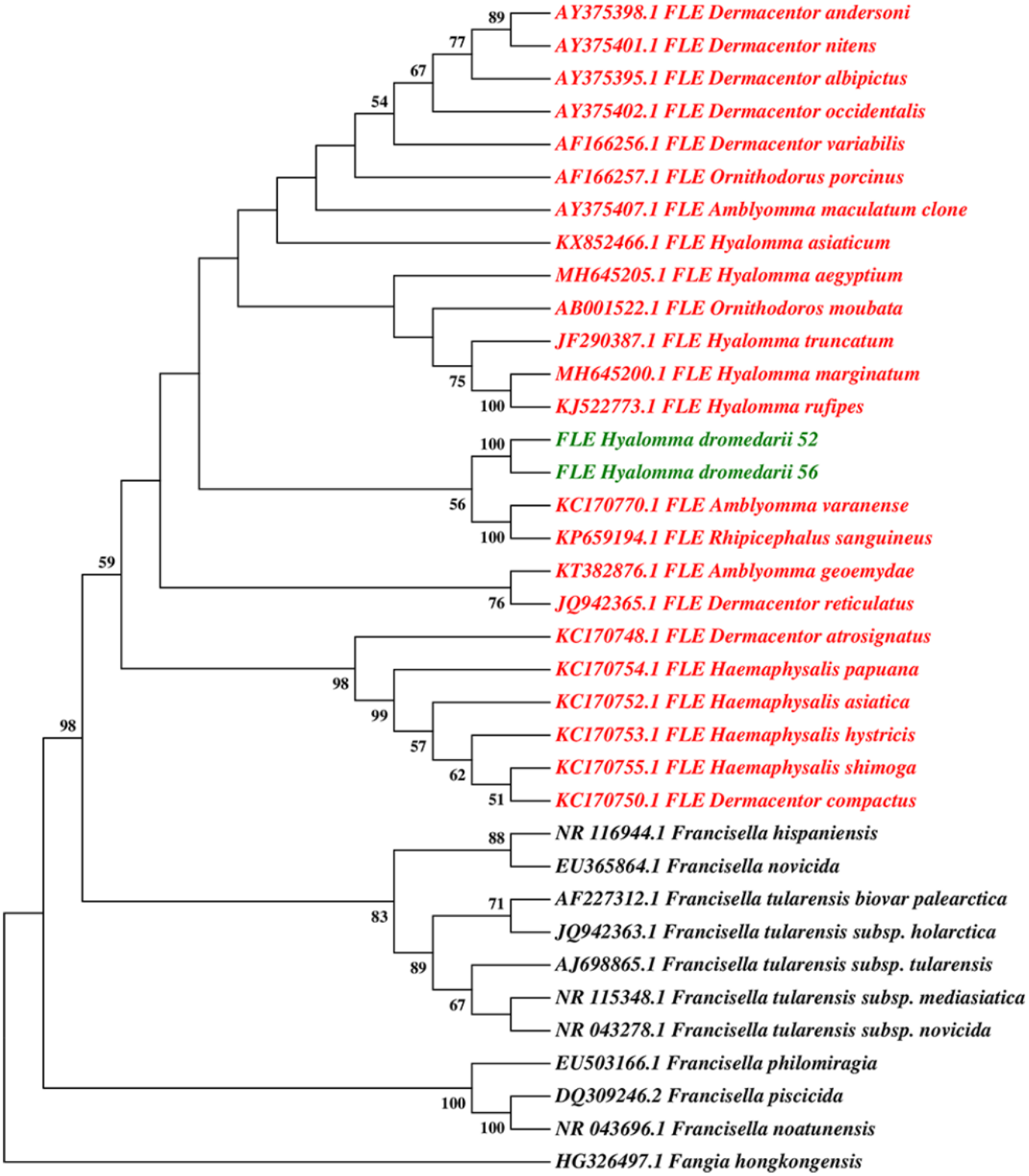
At the species level, thirty-three out of 114 genera contained more than one species, of these, *Corynebacterium*, *Bifidobacterium* and *Bacillus* genera constituted the most diverse genera, each containing 11 species. Other genera were listed in (Supplementary table 1, 2 and 3). Following the classification criteria adopted in the study, only 16 out of 217 unique OTUs could be classified to the species level (Table 3). Of these, 2 out of 3 OTUs of the genus *Helicobacter* were classified as *Helicobacter pylori* with similarity value of 99.7% to *H. pylori* in GenBank database and had a distance of more than 1 to *Helicobacter cetorum* and *H. pullorum* with 98.1% identity. The V3-V4 16S rRNA sequence phylogeny clustered *H. pylori* sequence found in the study with Genbank *H. pylori* sequences, while separated from other *Helicobacter* species (Figure 2, Supplementary Figure 1) The other OTU of the genus *Helicobacter* was unclassifiable.

Unfortunately, the *Francisella* V3-V4 region of 16S rRNA gene matches best to *Francisella*-like endosymbiont (FLE) of *Hyalomma marginatum* at 98.71% identity, which is below the threshold 99.3% for species assignment adopted in this study. To get better taxonomic resolution a 1071bp region of *Francisella* sp. 16S rRNA gene was amplified and sanger sequenced. BLASTn results showed 98.77, 98.68, 98.59, 98.30% similarity to FLEs of *D. auratus* (JQ764629.1), FLEs of *Ornithodoros moubata* (AB001522.1), FLEs of *Hyalomma asiaticum* (KX852466.1) and *Francisella hispaniensis* (CP018093.1) respectively. As for *Francisella tularensis* strains, the similarity varied from 98.11% to 97.9%. The phylogenetic inference based on 1071bp 16S rRNA gene sequence showed that *Francisella* sp. was genetically related to others symbiotic *Francisella*, while separated from the others pathogenic *Francisella* species (Figure 3, Supplementary Figure 2).

Table 2. Relative abundance of tick bacteria phyla classified in pooled ticks: 28, 29, 30, 55, 64, 65, 71, 73.01 and individual ticks 96, 100. Values were presented as %.

Phylum	Tick library name									
	28	29	30	55	64	65	71	73_1	100	96
Actinobacteria	0.18	0.16	0.33	0.01	0.58	0.16	0.16	0.51	0.91	0.32
Bacteroidetes	0.04	0.05	0.2	0	0	0.04	0.02	0.11	0.23	0.9
Cyanobacteria	0	0.04	0.01	0	0.07	0.01	0.11	0.01	0	0
Firmicutes	1.75	0.67	1.82	0.23	3.05	0.82	0.81	1.53	1.36	1.31
Proteobacteria	98.03	99.02	97.58	99.76	96.3	98.97	98.9	97.72	97.5	97.36
Verrucomicrobia	0	0	0.01	0	0	0	0	0.03	0	0
Unclassified	0	0.05	0.03	0	0	0	0.01	0.09	0	0.11

Figure 3. Maximum-likelihood tree based on 1071bp 16S rRNA gene sequences of FLE *Hyalonma dromedarii* (sample no 52 and 56) generated as part of this study and selected sequences of *Francisella* species from the GenBank. Analysis was conducted in MEGA7 [58] based on Tamura-Nei model with 100 bootstraps. Only Bootstrap values > 50% are shown.



In addition, *Francisella* sp. grouped with the FLEs of *Amylyomma varanense* and *Rhipicephalus sanguineus* rather than with the closely clade containing *Hyalomma rufipes*. Based on these results we classify *Francisella* sp. as FLE of *H. dromedarii*.

Prevalence rate of bacteria among pooled samples

The whole *H. dromedarii* bacteriome at the genus and species level is divided into; 1) highly prevalent bacteria which is defined as genera or species found in all eight pooled samples of tick species. At the genus level, the highly prevalent bacteria consisted of four genera (*Francisella, Salinicoccus, Corynebacterium* and *Staphylococcus*) representing 95.8% of average sequences (Supplementary Table 1), while at the species level consisted of FLE of *H. dromedarii* and *Salinicoccus* sp. accounting for 94.51% of average sequences. 2) Moderately prevalent bacterial genera having prevalence of 50 to 90%(Supplementary Table 2). It accounted for 1.15% of average sequences and 24.42% (53 species) of total bacteria and 3) low prevalent bacterial genera having prevalence less than 40%, which represent 3.5% of average sequences and constituted 66.36% (144 species) of total bacterial (Supplementary Table 3).

Discussion

In this study, we conducted a cross sectional survey for bacterial community in whole *H. dromedarii* ticks in Saudi Arabia. The bacteriome of *H. dromedarii* ticks was analyzed via sequencing of the V3-V4 segment of 16S rRNA gene using Illumina MiSeq sequencer. Although ticks were randomly collected from camel, *H. dromedarii* ticks are the only ticks found during collection. This finding is consistent with previous report revealing the dominance of *H. dromedarii* ticks in camels of Saudi Arabia [3].

Using this approach, a total of 6 phyla were detected in tick *H. dromedarii* and *Proteobacteria* was the most abundant which agreed with the composition of bacterial community reported in several tick species [20]. Although *Proteobacteria* is more dominant at sequences level than *Firmicute*s phylum, the number of genera assigned to *Firmicutes* (214 OTUs) surpassed the number of genera assigned to *Proteobacteria* (160 OTUs). Furthermore, *Proteobacteria, Firmicutes* and *Actinobacteria* phyla were prevalent in all samples either in individual or pooled samples.

Several human pathogens were detected in this study such as *Helicobacter pylori*, the causative agents of stomach peptic ulcer disease in human. A disease proposed to spread among human through the oral-oral or fecal-oral routes [21]. *H. pylori* were detected in 3 tick pools (30%). However, the presence of *H. pylori* in *H. dromedarii* ticks does not prove that the ticks act as reservoir or competent vector, but remains to be elucidated. It also raises a question how *H. dromedarii* ticks acquire this bacterium. Previous studies reported *H. pylori* in the stomach of domestic animals without having gastritis [22,23] and also found in the milk of camel, cow and goat. Therefore, proper caution is required when removing or handling ticks during collection to avoids hand contamination. To our knowledge, this is first report of *H. pylori* DNA in ticks, but non-*H. pylori* species such as *Helicobacter bizzozeronii* was detected before in *H. rufipes* ticks in china [7].

Table 3. list of OTUs classified to species level using V3-V4 region of 16S rRNA gene.

Species	Habitat/Medial importance	References
Helicobacter pylori	Human gastric ulcer	21
Corynebacterium confusum	foot infections in human	26
Corynebacterium massiliense	Isolated from human hip joint fluid	27
Granulicatella adiacens	human mucosal surfaces commensal	31
Akkermansia muciniphila	Human intestinal tract commensal and many other animals.	32
Anaerostipes hadrus	Human colonic microbiota	33
Nocardioides islandensis	Isolated from soil	41
Sporaceti₂enium mesophilum	Isolated from solid waste and sewage	42
Devosia albogilva	Isolated from a hexachlorocyclohexane dump site in India	43
Lysobacter defluvii	isolated from municipal solid waste	44
Acinetobacter schindleri	Isolated from bacteraemia in an immunocompromised patient	49
Acinetobacter variabilis	Human Leg wound, urine, faeces and blood	50
Acinetobacter radioresistens	Human skin commensal, opportunistic pathogen. Isolated from cotton and soil.	51
Bifidobacterium breve	Human gut microbiota	52
Salinicoccus kunmingensis	Isolated from a brine sample from a salt mine	53
Bacillus malikii	Isolated from tannery effluent wastewater	54

Table 4. Genera prevalence among bacterial community of ticks detected from whole body (W) and internal organs (Int) from 12 tick species.

Species	**H. r**	**H. a**	**H. m**	**H. e**	**I. r**	**I. o**	**I. p**	**R. m**	**H. f**	**R. s**	**A.t**	**I. h**	
Body site	**W**	**W**	**W**	**W**	**W**	**Int**	**Int**	**Int**	**Int**	**W**	**W**	**W**	**Prev**
Reference	**[7]**	**[9]**	**[9]**	**[9]**	**[11]**	**[23]**	**[25]**	**[24]**	**[48, 23]**	**[55]**	**[56]**	**[57]**	
Bacillus	P	P	P	P	P			P		P	P	P	9
Staphylococcus		P				P	P	P	P	P	P	P	8
Corynebacterium	P					P	P	P	P		P	P	7
Pseudomonas	P					P	P	P	P	P	P		7
Acinetobacter	P					P	P		P	P	P		6
Ralstonia			P	P		P	P				P	P	6
Bradyrhizobium						P	P		P	P	P		5
Clostridium	P		P		P			P			P		5
Enterococcus						P	P	P		P	P		5
Escherichia	P		P	P	P			P					5
Francisella		P	P	P							P	P	5
Lactobacillus	P				P	P	P			P			5
Limnohabitans			P	P		P	P		P				5
Massilia			P				P		P	P	P		5
Streptococcus						P	P	P	P			P	5
Comamonas						P	P	P			P		4
Methylobacterium						P	P		P		P		4
Paracoccus						P	P				P	P	4
Prevotella				P	P			P				P	4
Bacteroides			P		P			P					3
Brachybacterium							P	P			P		3
Brevibacterium							P	P			P		3
Brevundimonas							P	P	P				3
Caulobacter						P	P	P					3
Ruminococcus	P		P				P						3
Acidaminococcus	P	P											2
Atopostipes							P		P				2
Devosia							P		P				2
Dietzia							P				P		2
Granulicatella						P	P						2
Janthinobacterium			P				P						2
Lysobacter					P						P		2
Macrococcus									P	P			2
Pantoea								P			P		2
Peptoniphilus							P		P				2
Anaerostipes								P					1
Citrobacter	P												1
Dorea								P					1
Enterobacter								P					1
Halomonas							P						1
Klebsiella											P		1
Luteolibacter							P						1
Lysinibacillus										P			1
Paraprevotella							P						1
Planococcus											P		1
Proteus										P			1
Schlegelella											P		1
Sphingobacterium											P		1
Turicibacter								P					1
Helicobacter	P												1
Porphyromonas							P						1

W: whole body, Int: internal organs, R. m: Rhipicephalus (Boophilus) microplus; R. s: *Rhipicephalus sanguineus,* I. r: *Ixodes Ricinus*, I. h: *Ixodes holocyclus*, A. t: *Amblyomma tuberculatum*, I. o: *Ixodes ovatus*, I. p: *Ixodes persulcatus*, H. f: *Haemaphysalis flava*, H. r: *Hyalomma rufipes*, H. a: *Hyalomma aegyptium*, H. m: *Hyalomma marginatum*, H. e: *Hyalomma excavatum,* P: indicate presence of bacteria and empty cell: indicate absence of bacteria; Prev: Prevalence.

Among high prevalent genera, the potential pathogenic genera *Staphylococcus* and *Corynebacterium* were similarly detected in *Rhipicephalus microplus*, *Rhipicephalus sanguineus*, *Ixodes ricinus*, *Ixodes holocyclus*, *Amblyomma tuberculatum*, *Ixodes ovatus*, *Ixodes persulcatus*, *Haemaphysalis flava*, *Hyalomma rufipes*, *Hyalomma aegyptium* , *Hyalomma marginatum* and *Hyalomma excavatum* (Table 4). Notably, *Staphylococcus* and *Corynebacterium* have previously been detected from the saliva content of *H. flava* [24]. Among moderately prevalent genera we also found *Pseudomonas* and *Enterococcus* which were detected previously from internal organ of *Rhipicephalus* (*Boophilus*) *microplus*, *Ixodes ovatus* and *I. persulcatus* [25]. Thus, these genera are probably true members of tick bacteriome. Furthermore, the species *Acinetobacter variabilis*, *Acinetobacter schindleri*, *Corynebacterium confusum* and *Corynebacterium massiliense* have been detected before in human clinical specimens [26,27]. Among low prevalent genera we also found pathogenic genera such as *Klebsiella, Lactococcus, Lysinibacillus* and *Massilia* that have species previously been detected in human clinical specimens [28-30]. The lower prevalence and abundance of pathogenic genera suggest they are likely transient bacteria acquired from surrounding environment. Other bacterial species were commensal of human intestinal tract, such as, *Granulicatella adiacens*, *Akkermansia muciniphila* and *anaerostipes hadrus* that is found in human colon (Table 3) [31-33].

Coxiella burnetii is the etiological agent for Q fever, a worldwide zoonotic disease reported in human and animals such as camels, sheep, goats and cattle [34]. Transmission of Q fever to animals via tick bite in nature has not been confirmed, yet ticks have been experimental shown to be competent vectors for the transmission of *C. burnetii* to animal hosts [35]. However, the presence of *C. burnetii* in *H. dromedarii* and some other ticks suggest a role for ticks in the epidemiology Q fever. Most noticeable in our survey is the absence of the genus *Coxiella* in our bacteriome analysis although the infection is common in camels in Saudi Arabia [36]. The other noteworthy tick-borne pathogens found previously in *H. dromedarii* ticks outside Saudi Arabia but not found in our bacteriome analysis include *Rickettsia, Anaplasma* and *Ehrlichia* genera [6, 37]. It is possible that these pathogens are absent in *H. dromedarii* tick population in Saudi Arabia, or have low prevalence, thus not detected here because of the small sample size.

Endosymbiotic bacteria inhabit several tick species and most frequently predominant bacterial community [38]. FLE of *H. dromedarii* detected herein was the most abundant bacteria present in all samples and accounted for 94% of average sequences. Our finding is similar to a recent study showing that *Francisella* constituted 92.1% of relative percent abundance in *Hyalomma aegyptium* ticks [9]. Although the reasons that causes FLE of *H. dromedarii* to be the most abundant is lacking, Duron et al., 2018 [39] showed that FLE of *O. moubata* synthesize B vitamins that are missing in the blood meal of ticks and experimental removal of FLE of *O. moubata* restrain ticks' growth. Hence, previous findings suggest a possible symbiotic relationship between *Francisella* and *H. dromedarii* tick. Notably, most members of the genus *Francisella* are pathogenic. However, the pathogenicity of FLE of *H. dromedarii* remains unknown.

In addition to pathogenic and endosymbiotic bacteria, environment associated bacteria were observed including the soil bacteria *Solibacillus* which was isolated previously from the midgut of sand flies [40] and salt mine associated *Salinicoccus kunmingensis*. Other soil members among low prevalent genera comprise the genera *Pusillimonas, Oxalicibacterium* and *N. islandensis* [41]. Furthermore, *S. mesophilum* and *D albogilva* and *L. defluvii* [42-44] were reported from wastewater. Although the detected genera *Lysobacter, Pantoea, Paracoccus, Pontibacter* and *Pseudomonas* are frequent members of sandy soil of Saudi Arabia, the current study has yet to determine that these genera are acquired from the environment [45]. Finally, the presence of 12 unclassified OTUs may indicate the existence of as yet uncharacterized novel species.

The high prevalent bacterial genera (*Francisella, Salinicoccus, Corynebacterium* and *Staphylococcus*) probably encodes certain functions associated with tick survival and reproduction, which warrants further investigation to elucidate. Tick as external parasite can acquire bacteria from host skin or environment as shown previously for *H. dromedarii* ticks [46]. Hence, environmental factors may shape *H. dromedarii* bacteriome. In sum, our review of 12-tick species microbiota reveals that the genera *Bacillus, Staphylococcus Corynebacterium and Pseudomonas* are highly prevalent in ticks. Furthermore, 46.5 % (53 genera) of bacteria found in the present study have been detected previously from other tick species (Table 4).

As for bacterial abundance, FLE of *H. dromedarii* was the most abundant coexisting bacteria present in all samples, with abundance ranged from 93% to 99% of

sequence reads in pooled samples except sample 55 having abundance of 70%, while in individual samples the abundance was 95.6 and 96.6% in, Other genera with abundance above 1% include *Proteus* (29.7%), *Acinetobacter* (3.38%) and *Staphylococcus* (2.22%), while the remainder of detected bacterial genera had abundance less than 1%. The noteworthy in sample 55 it exhibits the lowest species richness (26), high abundance of Proteus (29.70%) and low abundance of FLE of *H. dromedarii* (70.03%), contrary to high abundance of FLE of *H. dromedarii* (> 93%) in the rest of samples. Although this observation warrants some sort of correlation analysis of absolute abundances across individuals to explain this finding, previous studies have demonstrated that tick bacteriome can interfere with pathogens colonization and transmission. For instance, in *Dermacentor andersoni* ticks, a reduction in *Francisella* endosymbionts was associated with lower *Francisella novicida* abundance levels [47]. In addition, an increase in ratio of *Rickettsia bellii* was associated with reduction of *Anaplasma marginale* levels in *Dermacentor andersoni* ticks [47]. Furthermore, a study reported that *Ixodes scapularis* microbiome composition could influence *Borrelia burgdorferi* colonization [48].

One of the limitations of our study it focused on whole tick bacteriome, therefore it did not differentiate between internal bacteria of ticks and the bacterial species residing on the exoskeleton. Regardless of this, 35.1% of genera detected in the current study have been reported from internal organs (saliva, midgut and ovaries) of several ticks (Table 4). On the other hand, biologically transmission is not the sole route of pathogen transmission; non-salivary mechanical bacterial transmission can also occur by contamination of injuries induced at feeding site with exoskeleton bacteria, raising the importance of exoskeleton bacteria. Another limitation of our study is small sample size, which prevents us from confirming the presence or absence of some tick-associated pathogens such as *Rickettsia*, *Anaplasma* and *Ehrlichia*.

Conclusion

This study has characterized the bacteriome of whole *H. dromedarii* ticks and revealed that the ticks mainly colonized by *Francisella* endosymbionts with low abundance of potential pathogens and environmental bacteria. However, pathogens detected herein do not indicate that *H. dromedarii* is a competent vector but may pose potential risk to humans and animals. The results presented here expanded our knowledge of the bacteria present in *H. dromedarii* ticks which provides a starting step for future comprehensive pathogens surveillance of *H. dromedarii*. Furthermore, our finding opens a new avenue of research to study the role of *H. dromedarii* in the epidemiology of *H. pylori* and the impact of FLEs on the colonization and transmission of bacteria in ticks.

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References

1. Salih DA, Hassan SM, El Hussein AM, Jongejan F (2004) Preliminary survey of ticks (Acari: *Ixodidae*) on cattle in northern Sudan. Onderstepoort J Vet Res 71: 319-326.
2. El-Azazy OM, Scrimgeour EM (1997) Crimean-Congo haemorrhagic fever virus infection in the western province of Saudi Arabia. Trans R Soc Trop Med Hyg 91: 275-278.
3. Champour M, Chinikar S, Mohammadi G, Razmi G, Shah-Hosseini N, Khakifirouz S, Mostafavi E, Jalali T (2016) Molecular epidemiology of Crimean-Congo hemorrhagic fever virus detected from ticks of one humped camels (*Camelus dromedarius*) population in northeastern Iran. J Parasit Dis 40: 110-115.
4. Mahdi M, Erickson BR, Comer JA, Nichol ST, Rollin PE, AlMazroa MA, Memish ZA (2011) Kyasanur Forest Disease virus Alkhurma subtype in ticks, Najran Province, Saudi Arabia. Emerg Infect Dis 17: 945-947.
5. Nassar AM (1992) *Theileria* infection in camels (*Camelus dromedarius*) in Egypt. Vet Parasitol 43: 147-149.
6. Kernif T, Djerbouh A, Mediannikov O, Ayach B, Rolain JM, Raoult D, Parola P, Bitam I (2012) *Rickettsia africae* in *Hyalomma dromedarii* ticks from sub-Saharan Algeria. Ticks Tick Borne Dis 3: 377-379.
7. Luo J, Liu MX, Ren QY, Chen Z, Tian ZC, Hao JW, Wu F, Liu XC, Luo JX, Yin H, Wang H, Liu GY (2017) Micropathogen Community Analysis in *Hyalomma rufipes* via High-Throughput Sequencing of Small RNAs. Front Cell Infect Microbiol 7: 374.
8. Karim S, Budachetri K, Mukherjee N, Williams J, Kausar A, Hassan MJ, Adamson S, Dowd SE, Apanskevich D, Arijo A, Sindhu ZU, Kakar MA, Khan RMD, Ullah S, Sajid MS, Ali A, Iqbal Z (2017) A study of ticks and tick-borne livestock pathogens in Pakistan. PLoS Negl Trop Dis 11:e0005681.
9. Keskin A, Bursali A, Snow DE, Dowd SE, Tekin S (2017) Assessment of bacterial diversity in *Hyalomma aegyptium, H. marginatum and H. excavatum* ticks through tag-encoded pyrose quencing. Exp Appl Acarol 73: 461-475.
10. Williams-Newkirk AJ, Rowe LA, Mixson-Hayden TR, Dasch GA (2014) Characterization of the bacterial communities of life stages of free living lone star ticks (*Amblyomma americanum*). PLoS One 9 :e102130.
11. Carpi G, Cagnacci F, Wittekindt NE, Zhao F, Qi J, Tomsho LP, Drautz DI, Rizzoli A, Schuster SC (2011) Metagenomic profile of the bacterial communities associated with Ixodes ricinus ticks. PLoS One 6 :e25604.

12. Lv J, Wu S, Zhang Y, Chen Y, Feng C, Yuan X, Jia G, Deng J, Wang C, Wang Q, Mei L, Lin X. (2014) Assessment of four DNA fragments (COI, 16S rDNA, ITS2, 12S rDNA) for species identification of the *Ixodida* (Acari: *Ixodida*). Parasit Vectors 7: 93.
13. Barns SM, Grow CC, Okinaka RT, Keim P, Kuske CR (2005) Detection of diverse new *Francisella*-like bacteria in environmental samples. Appl Environ Microbiol 71: 5494-5500.
14. Klindworth A, Pruesse E, Schweer T, Peplies J, Quast C, Horn M, Glöckner FO (2013) Evaluation of general 16S ribosomal RNA gene PCR primers for classical and next-generation sequencing-based diversity studies. Nucleic Acids Res ; 41:e1.
15. Magoč T, Salzberg SL (2011) FLASH. fast length adjustment of short reads to improve genome assemblies. Bioinformatics 27: 2957-2963.
16. Caporaso JG, Kuczynski J, Stombaugh J, Bittinger K, Bushman FD, Costello EK, Fierer N, Peña AG, Goodrich JK, Gordon JI, Huttley GA, Kelley ST, Knights D, Koenig JE, Ley RE, Lozupone CA, McDonald D, Muegge BD, Pirrung M, Reeder J, Sevinsky JR, Turnbaugh PJ, Walters WA, Widmann J, Yatsunenko T, Zaneveld J, Knight R (2010) QIIME allows analysis of high-throughput community sequencing data. Nat Methods 7: 335-336.
17. Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ (1990) Basic local alignment search tool. J Mol Biol 5: 403-10.
18. Li W, Fu L, Niu B, Wu S, Wooley J (2012) Ultrafast clustering algorithms for metagenomic sequence analysis. Brief Bioinform 13: 656-668.
19. Villmones HC, Haug ES, Ulvestad E, Grude N, Stenstad T, Halland A, Kommedal Ø (2018) Species Level Description of the Human Ileal Bacterial Microbiota. Sci Rep 8: 4736.
20. Portillo A, Palomar AM, de Toro M, Santibáñez S, Santibáñez P, Oteo JA (2019) Exploring the bacteriome in anthropophilic ticks: To investigate the vectors for diagnosis. PLoS One 14: e0213384.
21. Bui D, Brown HE, Harris RB, Oren E (2016) Serologic Evidence for Fecal-Oral Transmission of *Helicobacter pylori*. Am J Trop Med Hyg 94: 82-88.
22. Dore MP, Sepulveda AR, El-Zimaity H, Yamaoka Y, Osato MS, Mototsugu K, Nieddu AM, Realdi G, Graham DY (2001) Isolation of *Helicobacter pylori* from sheep-implications for transmission to humans. Am J Gastroenterol 96: 1396-1401.
23. Rahimi E, Kheirabadi EK (2012) Detection of *Helicobacter pylori* in bovine, buffalo, camel, ovine, and caprine milk in Iran. Foodborne Pathog Dis 9: 453-456.
24. Qiu Y, Nakao R, Ohnuma A, Kawamori F, Sugimoto C. (2014) Microbial population analysis of the salivary glands of ticks; a possible strategy for the surveillance of bacterial pathogens. PLoS One 9: e103961.
25. Andreotti R, Perez AA, Dowd SE, Guerrero FD, Bendele KG, Scoles GA (2011) Assessment of bacterial diversity in the cattle tick *Rhipicephalus* (*Boophilus*) *microplus* through tag-encoded pyrosequencing. BMC Microbiol 11: 6.
26. Funke G, Osorio CR, Frei R, Riegel P, Collins MD (1998) *Corynebacterium confusum* sp. nov., isolated from human clinical specimens. Int J Syst Bacteriol 48: 1291-1296.
27. Merhej V, Falsen E, Raoult D, Roux V (2009) *Corynebacterium timonense* sp. nov. and *Corynebacterium massiliense* sp. nov., isolated from human blood and human articular hip fluid. Int J Syst Evol Microbiol 59: 1953-1959.
28. Chan JF, Woo PC, Teng JL, Lau SK, Leung SS, Tam FC, Yuen KY (2011) Primary infective spondylodiscitis caused by *Lactococcus garvieae* and a review of human *L. garvieae* infections. Infection 39: 259-264.
29. Wenzler E, Kamboj K, Balada-Llasat JM (2015) Severe Sepsis Secondary to Persistent *Lysinibacillus sphaericus*, *Lysinibacillus fusiformis* and *Paenibacillus amylolyticus* Bacteremia. Int J Infect Dis 35: 93-95.
30. Lindquist D, Murrill D, Burran WP, Winans G, Janda JM, Probert W (2003) Characteristics of *Massilia timonae* and *Massilia timonae*-like isolates from human patients, with an emended description of the species. J Clin Microbiol 41: 192-196.
31. Gardenier JC, Hranjec T, Sawyer RG, Bonatti H (2011) *Granulicatella adiacens* bacteremia in an elderly trauma patient. Surg Infect (Larchmt) 12: 251-253.
32. de Vos WM (2017) Microbe Profile: Akkermansia muciniphila: a conserved intestinal symbiont that acts as the gatekeeper of our mucosa. Microbiology 163: 646-648.
33. Allen-Vercoe E, Daigneault M, White A, Panaccione R, Duncan SH, Flint HJ, O'Neal L, Lawson PA (1976) *Anaerostipes hadrus* comb. nov., a dominant species within the human colonic microbiota; reclassification of *Eubacterium hadrum* Moore et al. 1976. Anaerobe 18: 523-529.
34. Meadows S, Jones-Bitton A, McEwen SA, Jansen J, Patel SN, Filejski C, Menzies P (2016) *Coxiella burnetii* (Q Fever) Seropositivity and Associated Risk Factors in Sheep and Goat Farm Workers in Ontario, Canada. Vector Borne Zoonotic Dis 16: 643-649.
35. Siroký P, Kubelová M, Modrý D, Erhart J, Literák I, Spitalská E, Kocianová E (2010) Tortoise tick *Hyalomma aegyptium* as long term carrier of Q fever agent *Coxiella burnetii*--evidence from experimental infection. Parasitol Res 107: 1515-1520.
36. Khalafalla AI, AlEknah MM, Abdelaziz M, Ghoneim IM (2017) A study on some reproductive disorders in dromedary camel herds in Saudi Arabia with special references to uterine infections and abortion. Trop Anim Health Prod 49: 967-974.
37. Jafar Bekloo A, Ramzgouyan MR, Shirian S, Faghihi F, Bakhshi H, Naseri F, Sedaghat M, Telmadarraiy Z (2018) Molecular Characterization and Phylogenetic Analysis of *Anaplasma* spp. and *Ehrlichia* spp. Isolated from Various Ticks in Southeastern and Northwestern Regions of Iran. Vector Borne Zoonotic Dis 18: 252-257.
38. Ahantarig A, Trinachartvanit W, Baimai V, Grubhoffer L (2013) Hard ticks and their bacterial endosymbionts (or would be pathogens). Folia Microbiol (Praha) 58: 419-428.
39. Duron O, Morel O, Noël V, Buysse M, Binetruy F, Lancelot R, Loire E, Ménard C, Bouchez O, Vavre F, Vial L (2018) Tick-Bacteria Mutualism Depends on B Vitamin Synthesis Pathways. Curr Biol 28: 1896-1902.
40. Dey R, Joshi AB, Oliveira F, Pereira L, Guimarães-Costa AB, Serafim TD, de Castro W, Coutinho-Abreu IV, Bhattacharya P, Townsend S, Aslan H, Perkins A, Karmakar S, Ismail N, Karetnick M, Meneses C, Duncan R, Nakhasi HL, Valenzuela JG, Kamhawi S (2018) Gut Microbes Egested during Bites of Infected Sand Flies Augment Severity of Leishmaniasis via Inflammasome-Derived IL-1β. Cell Host Microbe 23: 134-143.
41. Dastager SG, Lee JC, Ju YJ, Park DJ, Kim CJ (2008) *Nocardioides islandiensis* sp. nov., isolated from soil in Bigeum Island Korea. Antonie Van Leeuwenhoek 93: 401-406.
42. Chen S, Song L, Dong X (2006) *Sporacetigenium mesophilum* gen. nov., sp. nov., isolated from an anaerobic digester treating municipal solid waste and sewage. Int J Syst Evol Microbiol. 56: 721-725.

43. Verma M, Kumar M, Dadhwal M, Kaur J, Lal R (2009) *Devosia albogilva* sp. nov. and *Devosia crocina* sp. nov., isolated from a hexachlorocyclohexane dump site. Int J Syst Evol Microbiol 59: 795-799.
44. Yassin AF, Chen WM, Hupfer H, Siering C, Kroppenstedt RM, Arun AB, Lai WA, Shen FT, Rekha PD, Young CC (2007) *Lysobacter defluvii* sp. nov., isolated from municipal solid waste. Int J Syst Evol Microbiol 57: 1131-1136.
45. Eida AA, Ziegler M, Lafi FF, Michell CT, Voolstra CR, Hirt H, Saad MM (2018) Desert plant bacteria reveal host influence and beneficial plant growth properties. PLoS One 12: e0208223.
46. Koosha M, Vatandoost H, Karimian F, Choubdar N, Oshaghi MA (2018) Delivery of a Genetically Marked Serratia AS1 to Medically Important Arthropods for Use in RNAi and Paratransgenic Control Strategies. Microb Ecol doi:10.1007/s00248-018-1289-7.
47. Gall CA, Reif KE, Scoles GA, Mason KL, Mousel M, Noh SM, Brayton KA (2016) The bacterial microbiome of *Dermacentor andersoni* ticks influences pathogen susceptibility. ISME J 10: 1846-1855.
48. Narasimhan S, Rajeevan N, Liu L, Zhao YO, Heisig J, Pan J, Eppler-Epstein R, Deponte K, Fish D, Fikrig E (2014) Gut microbiota of the tick vector *Ixodes scapularis* modulate colonization of the Lyme disease spirochete. Cell Host Microbe 15: 58-71.
49. Montaña S, Palombarani S, Carulla M, Kunst A, Rodriguez CH, Nastro M, Vay C, Ramirez MS, Almuzara M (2017) First case of bacteraemia due to *Acinetobacter schindleri* harbouring bla(NDM-1) in an immunocompromised patient. New Microbes New Infect 16: 28-30.
50. Krizova L, McGinnis J, Maixnerova M, Nemec M, Poirel L, Mingle L, Sedo O, Wolfgang W, Nemec A (2015) *Acinetobacter variabilis* sp. nov. (formerly DNA group 15 sensu Tjernberg & Ursing), isolated from humans and animals. Int J Syst Evol Microbiol 65: 857-863.
51. Nishimura Y, Ino T, Iizuka H (2018) *Acinetobacter radioresistens* sp. nov. Isolated from Cotton and Soil. International Journal of Systematic Bacteriology 38: 209-211.
52. Bottacini F, O'Connell Motherway M, Kuczynski J, O'Connell KJ, Serafini F,Duranti S, Milani C, Turroni F, Lugli GA, Zomer A, Zhurina D, Riedel C, Ventura M, van Sinderen D (2014) Comparative genomics of the *Bifidobacterium breve* taxon. BMC Genomics 15: 1471-2164.
53. Chen YG, Cui XL, Pukall R, Li HM, Yang YL, Xu LH, Wen ML, Peng Q, Jiang CL (2007) *Salinicoccus kunmingensis* sp. nov., a moderately halophilic bacterium isolated from a salt mine in Yunnan, south-west China. Int J Syst Evol Microbiol 57: 2327-2332.
54. Abbas S, Ahmed I, Kudo T, Iqbal M, Lee YJ, Fujiwara T, Ohkuma M (2015) A heavy metal tolerant novel bacterium, *Bacillus malikii* sp. nov., isolated from tannery effluent wastewater. Antonie Van Leeuwenhoek 108: 1319-1330.
55. He XM, Cheng TY (2017) Tick saliva microbiomes isolated from engorged and partially fed adults of *Haemaphysalis flava* tick females. J Appl Entomol 142: 173-180.
56. René-Martellet M, Minard G, Massot R, Tran Van V, Valiente Moro C, Chabanne L, Mavingui P (2017) Bacterial microbiota associated with *Rhipicephalus sanguineus* (s.l.) ticks from France, Senegal and Arizona. Parasit Vectors 10: 416.
57. Budachetri K, Gaillard D, Williams J, Mukherjee N, Karim S (2016) A snapshot of the microbiome of *Amblyomma tuberculatum* ticks infesting the gopher tortoise, an endangered species. Ticks Tick Borne Dis 7: 1225-1229.
58. Kumar S, Stecher G, Tamura K (2016) MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets. Molecular Biology and Evolution 3: 1870-1874.

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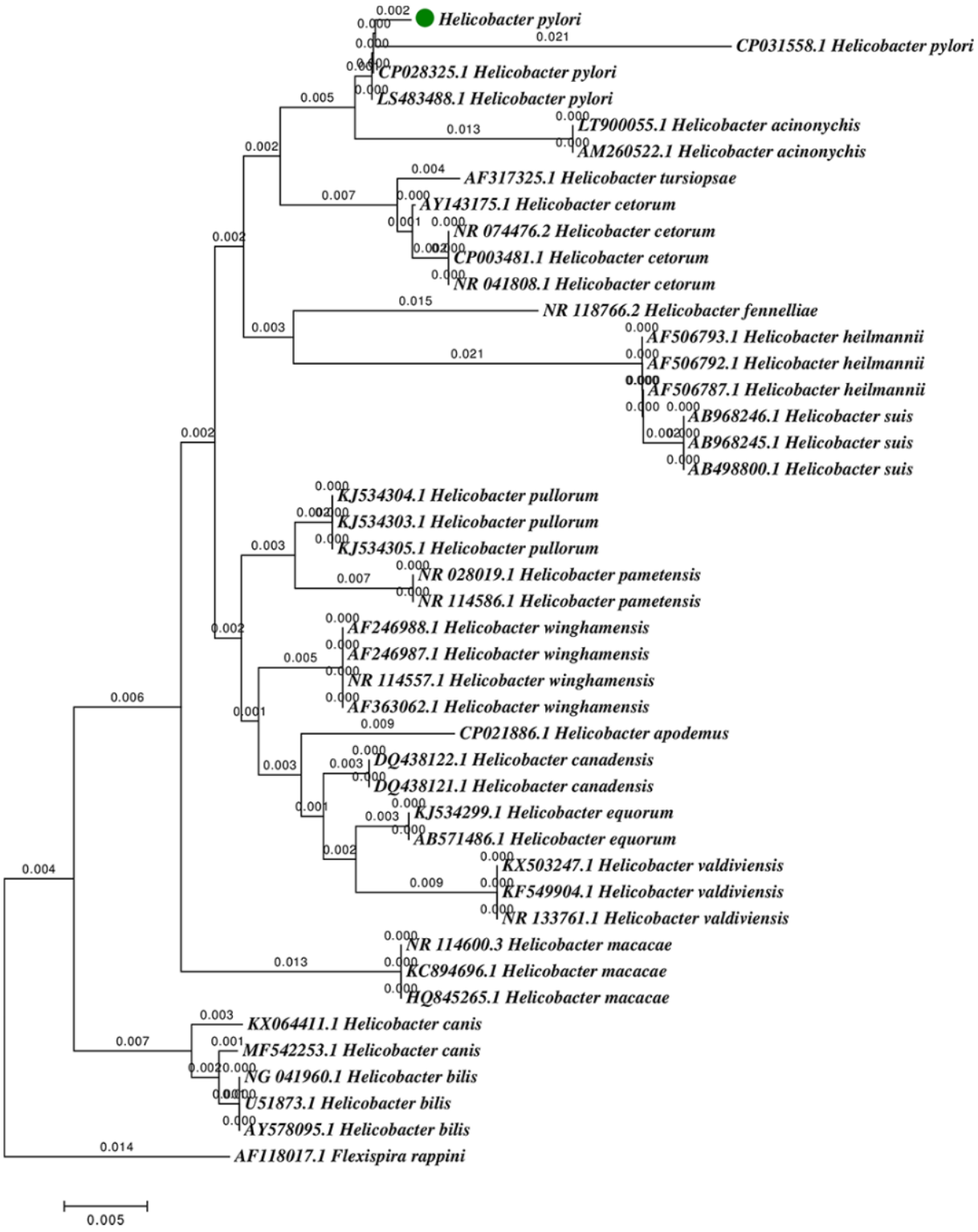
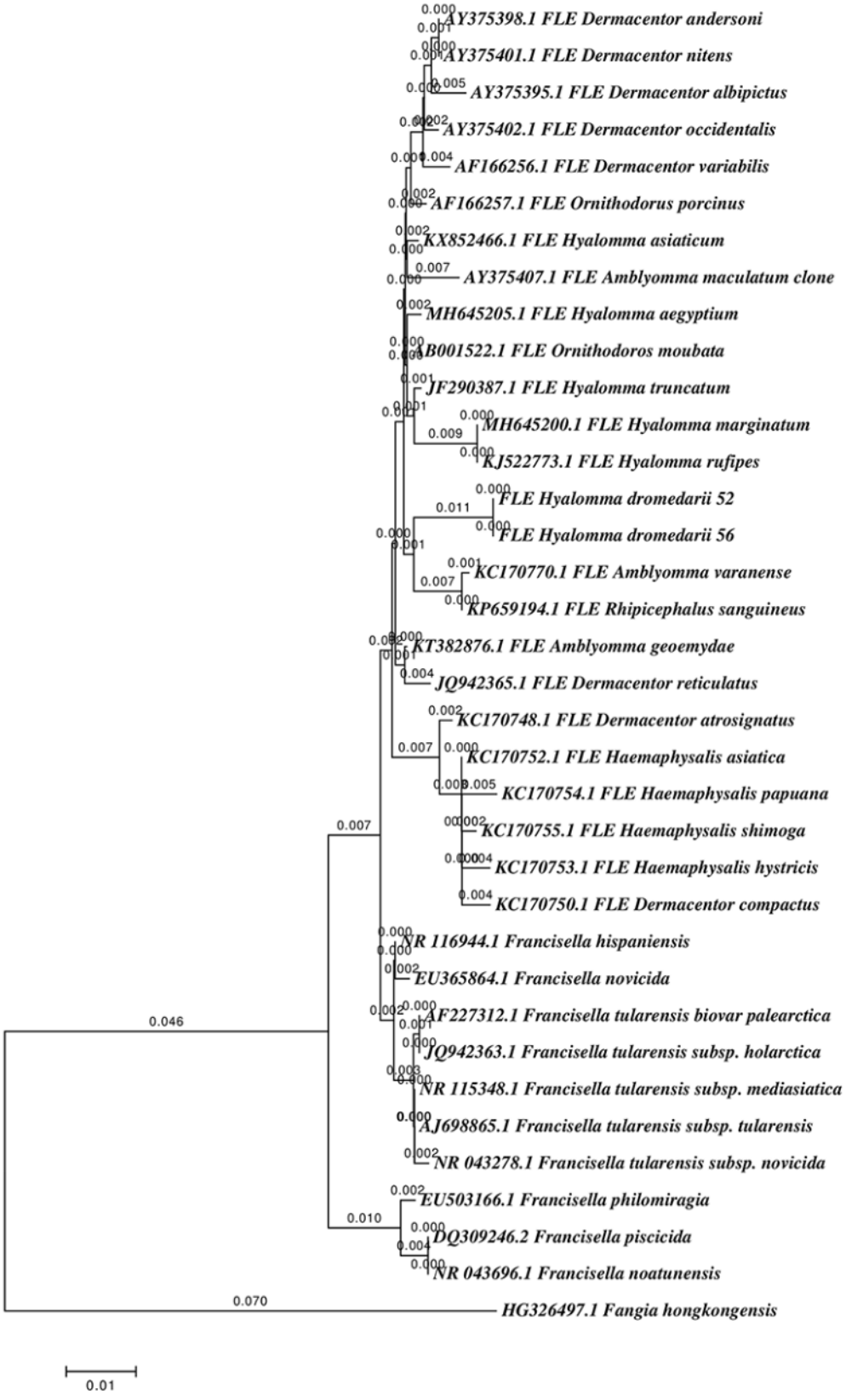
Annex – Supplementary Items

Supplementary Figure 1. Neighbor-Joining tree with branch lengths of the V3-V4 16S rRNA sequences of *Helicobacter* species and OTU identified as *Helicobacter pylori* (Green circle) in this study. The bootstrap consensus tree inferred from 100 replicates. Bootstrap values > 50% are shown. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Tamura-Nei method and are in the units of the number of base substitutions per site. The analysis involved 44 nucleotide sequences. All positions containing gaps and missing data were eliminated. There were a total of 431 positions in the final dataset. The analyses were conducted in MEGA7 [58].



Supplementary Figure 2. Maximum-likelihood tree with branch lengths based on 1071bp 16S rRNA gene sequences of FLE *Hyalomma dromedarii* (sample no 52 and 56) generated as part of this study and selected sequences of *Francisella* species from the GenBank. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site (next to the branches). The analysis involved 36 nucleotide sequences. There were a total of 1070 positions in the final dataset. Analysis was conducted in MEGA7 [58] based on Tamura-Nei model with 100 bootstraps.



Supplementary Table 1. Highly prevalent bacteria genera

Genus	28 (%)	29 (%)	30 (%)	55 (%)	64 (%)	65 (%)	71 (%)	73 (%)	100 (%)	96 (%)	Average abundance	Prevalence
Francisella	93.805	99.015	97.243	70.035	96.179	99.086	97.931	98.099	95.206	95.698	94.370	100%
Corynebacterium (11)	0.159	0.164	0.119	0.007	0.566	0.135	0.137	0.150	0.887	0.200	0.252	100%
Salinicoccus (5)	0.242	0.180	0.126	0.030	0.184	0.124	0.112	0.216	0.273	0.485	0.207	100%
Staphylococcus (3)	0.426	0.244	0.267	0.053	2.219	0.275	0.140	0.565	0.665	0.274	0.513	100%

Supplementary Table 2. Moderately prevalent bacterial genera.

Genus	28 (%)	29 (%)	30 (%)	55 (%)	64 (%)	65 (%)	71 (%)	73 (%)	100 (%)	96 (%)	Average abundance	Prevalence
Acinetobacter (8)	3.377	0.061	0.490	0.025	0.033	0.032	0.331	0.156	0.200	0.093	0.461	90%
Enterococcus (2)	0.100	0.145	0.357	0.037	0.327	0.091	0.053	0.063	0.059	0.000	0.123	90%
Pseudomonas (8)	0.515	0.029	0.054	0.002	0.008	0.008	0.219	0.000	0.000	0.528	0.136	80%
Pediococcus	0.038	0.005	0.000	0.000	0.004	0.004	0.020	0.116	0.024	0.235	0.045	80%
Solibacillus	0.483	0.000	0.108	0.005	0.035	0.045	0.160	0.000	0.004	0.137	0.099	80%
Psychrobacter (3)	0.227	0.004	0.021	0.070	0.054	0.024	0.261	0.000	0.069	0.000	0.073	80%
Bacillus (11)	0.032	0.000	0.000	0.002	0.131	0.009	0.145	0.000	0.022	0.095	0.044	70%
Pantoea	0.175	0.020	0.023	0.000	0.005	0.000	0.378	0.017	0.000	0.244	0.086	70%
Bacteroides (6)	0.011	0.027	0.081	0.000	0.000	0.000	0.017	0.000	0.110	0.000	0.025	50%
Turicibacter (2)	0.025	0.000	0.037	0.000	0.054	0.014	0.044	0.000	0.000	0.000	0.017	50%
Streptococcus (5)	0.006	0.000	0.031	0.000	0.000	0.042	0.003	0.207	0.000	0.000	0.029	50%
Clostridium (5)	0.000	0.030	0.008	0.000	0.009	0.018	0.000	0.035	0.000	0.000	0.010	50%
Dietzia (2)	0.014	0.000	0.000	0.000	0.001	0.007	0.005	0.002	0.000	0.000	0.003	50%

Supplementary Table 3. Low prevalent bacterial genera.

Genus	28 (%)	29 (%)	30 (%)	55 (%)	64 (%)	65 (%)	71 (%)	73 (%)	100 (%)	96 (%)	Average abundance	Prevalence
Blautia (7)	0.033	0.015	0.183	0.007	0.000	0.000	0.000	0.000	0.000	0.000	0.024	40%
Proteus	0.000	0.025	0.003	29.704	0.000	0.000	0.000	0.003	0.000	0.000	2.974	40%
Georgenia	0.000	0.000	0.000	0.000	0.000	0.011	0.000	0.000	0.003	0.112	0.013	30%
Salinibacterium	0.003	0.000	0.000	0.000	0.000	0.000	0.003	0.015	0.000	0.000	0.002	30%
Bifidobacterium (11)	0.000	0.000	0.186	0.000	0.000	0.000	0.008	0.093	0.000	0.000	0.029	30%
Prevotella (2)	0.013	0.000	0.009	0.000	0.000	0.000	0.000	0.000	0.040	0.000	0.006	30%
Alkalibacterium	0.000	0.000	0.006	0.000	0.001	0.000	0.000	0.015	0.000	0.000	0.002	30%
Lactobacillus (5)	0.000	0.000	0.077	0.000	0.000	0.000	0.000	0.000	0.139	0.011	0.023	30%
Paracoccus	0.004	0.000	0.000	0.007	0.010	0.000	0.000	0.000	0.000	0.000	0.002	30%
Helicobacter (2)	0.028	0.000	0.123	0.000	0.000	0.000	0.000	0.026	0.000	0.000	0.018	30%
Escherichia	0.000	0.000	0.014	0.000	0.000	0.000	0.000	0.000	0.621	0.176	0.081	30%
Halomonas (2)	0.128	0.000	0.000	0.000	0.000	0.000	0.000	0.006	0.000	0.048	0.018	30%
Lysobacter (2)	0.000	0.000	0.000	0.000	0.004	0.004	0.000	0.000	0.000	0.647	0.065	30%
Brachybacterium	0.000	0.000	0.000	0.000	0.004	0.000	0.000	0.011	0.000	0.000	0.001	20%
Citricoccus	0.000	0.000	0.000	0.000	0.005	0.000	0.000	0.002	0.000	0.000	0.001	20%
Salinimicrobium (6)	0.004	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.560	0.056	20%
Porphyromonas (3)	0.000	0.000	0.032	0.000	0.000	0.000	0.000	0.000	0.000	0.210	0.024	20%
Alistipes (3)	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.005	0.011	0.000	0.002	20%
Pontibacter (2)	0.010	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.037	0.005	20%
Echinicola	0.000	0.000	0.000	0.000	0.005	0.000	0.000	0.000	0.000	0.011	0.002	20%
Lysinibacillus (4)	0.015	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.099	0.000	0.011	20%
Jeotgalicoccus	0.000	0.000	0.010	0.000	0.000	0.000	0.005	0.000	0.000	0.000	0.001	20%
Macrococcus	0.000	0.000	0.004	0.000	0.000	0.000	0.000	0.000	0.023	0.000	0.003	20%
Atopostipes	0.000	0.000	0.000	0.000	0.019	0.000	0.000	0.009	0.000	0.000	0.003	20%
Lactococcus (2)	0.000	0.000	0.105	0.000	0.000	0.000	0.000	0.038	0.000	0.000	0.014	20%
Anaerostipes	0.013	0.000	0.017	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.003	20%
Caulobacter	0.000	0.000	0.000	0.000	0.010	0.000	0.005	0.000	0.000	0.000	0.001	20%

Genus	28 (%)	29 (%)	30 (%)	55 (%)	64 (%)	65 (%)	71 (%)	73 (%)	100 (%)	96 (%)	Average abundance	Prevalence
Devosia (2)	0.008	0.000	0.000	0.000	0.004	0.000	0.000	0.000	0.000	0.000	0.001	20%
Pusillimonas (2)	0.000	0.000	0.004	0.000	0.000	0.000	0.000	0.000	0.000	0.060	0.006	20%
Comamonas	0.000	0.000	0.005	0.000	0.000	0.000	0.000	0.000	0.000	0.004	0.001	20%
Massilia (3)	0.000	0.000	0.000	0.000	0.000	0.000	0.005	0.000	0.057	0.000	0.006	20%
Campylobacter	0.000	0.000	0.000	0.008	0.003	0.000	0.000	0.000	0.000	0.000	0.001	20%
Microbulbifer (2)	0.000	0.000	0.000	0.000	0.004	0.000	0.000	0.023	0.000	0.000	0.003	20%
Marinobacter (2)	0.000	0.000	0.004	0.000	0.000	0.000	0.000	0.000	0.000	0.008	0.001	20%
Akkermansia	0.000	0.000	0.010	0.000	0.000	0.000	0.000	0.026	0.000	0.000	0.004	20%
Exiguobacterium	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.017	0.039	0.006	20%
Brevibacterium	0.000	0.000	0.000	0.000	0.000	0.011	0.000	0.000	0.000	0.000	0.001	10%
Modestobacter	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.005	0.000	0.000	0.000	10%
Candidatus Limnoluna	0.000	0.000	0.004	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	10%
Nocardioides	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.006	0.000	0.000	0.001	10%
Herbidospora	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.018	0.000	0.000	0.002	10%
Aequorivita	0.000	0.000	0.024	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.002	10%
Sphingobacterium	0.000	0.008	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.001	10%
Barnesiella	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.026	0.000	0.000	0.003	10%
Parabacteroides	0.000	0.000	0.030	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.003	10%
Paraprevotella	0.000	0.000	0.000	0.000	0.000	0.038	0.000	0.000	0.000	0.000	0.004	10%
Cylindrospermum	0.004	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	10%
Peptoniphilus	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.009	0.000	0.000	0.001	10%
Allobaculum	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.009	0.000	0.000	0.001	10%
Terribacillus	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.005	0.000	0.000	0.000	10%
Brevibacillus	0.017	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.002	10%
Granulicatella	0.000	0.000	0.030	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.003	10%
Pediococcus	0.000	0.000	0.023	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.002	10%
Butyricicoccus	0.000	0.000	0.000	0.000	0.000	0.018	0.000	0.000	0.000	0.000	0.002	10%
Eubacterium (3)	0.000	0.000	0.046	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.005	10%
Dorea	0.000	0.000	0.013	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.001	10%
Roseburia (2)	0.000	0.000	0.027	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.003	10%
Desulfotomaculum	0.000	0.008	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.001	10%
Sporacetigenium	0.000	0.000	0.000	0.000	0.000	0.005	0.000	0.000	0.000	0.000	0.001	10%
Faecalibacterium	0.000	0.000	0.024	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.002	10%
Gemmiger	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.021	0.000	0.000	0.002	10%
Ruminococcus	0.000	0.011	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.001	10%
Sporobacter	0.000	0.000	0.004	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	10%
Acidaminococcus	0.000	0.000	0.008	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.001	10%
Brevundimonas	0.000	0.000	0.000	0.000	0.000	0.000	0.016	0.000	0.000	0.000	0.002	10%
Phenylobacterium	0.000	0.000	0.004	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	10%
Bradyrhizobium	0.000	0.004	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	10%
Methylobacterium	0.000	0.000	0.000	0.000	0.004	0.000	0.000	0.000	0.000	0.000	0.000	10%
Venzinia	0.000	0.000	0.000	0.000	0.000	0.000	0.005	0.000	0.000	0.000	0.000	10%
Erythrobacter	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.008	0.000	0.000	0.001	10%
Ralstonia	0.000	0.000	0.000	0.000	0.004	0.000	0.000	0.000	0.000	0.000	0.000	10%
Desulfovibrio	0.000	0.004	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	10%
Enterobacter	0.014	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.001	10%
Klebsiella	0.074	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.007	10%
Oleiphilus	0.000	0.000	0.000	0.005	0.000	0.000	0.000	0.000	0.000	0.000	0.000	10%
Saccharospirillum	0.000	0.000	0.004	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	10%
Haemophilus	0.000	0.000	0.000	0.000	0.013	0.000	0.000	0.000	0.000	0.000	0.001	10%
Chthoniobacter	0.000	0.000	0.004	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	10%
Luteolibacter	0.000	0.000	0.000	0.005	0.000	0.000	0.000	0.000	0.000	0.000	0.000	10%
Citrobacter	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.010	0.000	0.001	10%
Desemzia	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.024	0.002	10%
Dialister	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.006	0.000	0.001	10%
Dolosigranulum	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.007	0.000	0.001	10%

Genus	28 (%)	29 (%)	30 (%)	55 (%)	64 (%)	65 (%)	71 (%)	73 (%)	100 (%)	96 (%)	Average abundance	Prevalence
Flaviramulus	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.071	0.000	0.007	10%
Janthinobacterium	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.038	0.000	0.004	10%
Jonesia	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.011	0.000	0.001	10%
Limnohabitans	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.021	0.000	0.002	10%
Nesterenkonia	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.004	0.000	0.000	10%
Nitriliruptor	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.008	0.001	10%
Olivibacter	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.008	0.001	10%
Oxalicibacterium	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.060	0.000	0.006	10%
Roseivirga	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.028	0.003	10%
Schlegelella	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.014	0.000	0.001	10%
Steroidobacter	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.010	0.000	0.001	10%
Succiniclasticum	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.015	0.000	0.001	10%
Syntrophococcus	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.004	0.000	0.000	10%
Virgibacillus	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.004	0.000	10%