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Metagenomic Nanopore Sequencing of Tickborne Pathogens, Mongolia

Appendix 1

Appendix 1 Table 1. Distribution and species composition of tick and processing approach according to sampling sites. Where multiple samples were pooled, numbers of specimens are listed followed by numbers of pools given in parentheses.

i	Dermac	entor nuttalli	Hyalomma asiaticum		Ixodes persulcatus		Total no.
Location	Single	Pooled (no.)	Single	Pooled (no.)	Single	Pooled (no.)	(%)
Site 1 (Lat. 44.492,	NA	NA	100	NA	NA	NA	100 (11.2)
Long.105.542)							
Site 2 (Lat. 44.490,	NA	NA	NA	176 (15)	NA	NA	176 (19.8)
Long.105.531)							
Site 3 (Lat. 49.774, Long.	NA	NA	NA	NA	188	156 (15)	344 (38.8)
107.604)							
Site 4 (Lat. 46.91, Long. 99.151)	89	36 (3)	NA	NA	NA	NA	125 (14.1)
Site 5 (Lat. 46.03, Long. 100.852)	NA	36 (3)	NA	NA	NA	NA	36 (4)
Site 6 (Lat. 48.415, Long. 90.986)	NA	104 (9)	NA	NA	NA	NA	104 (11.7)
Total count (%)	265 (29.9)		276 (31.1)		344 (38.8)		885
Total pools	15		15		15		45

*Lat., latitude; Long., longitude; NA, not applicable.

			Hyalomma		Ixodes		
	Dermacentor nuttalli		asiaticum		persulcatus		
		Pooled,	Single,	Pooled,	Single,	Pooled,	Total no.
Virus	Single, n = 2	n = 15	n = 11	n = 15	n = 10	n = 11	(%)
Bole tick virus 3	NA	NA	NA	10	NA	NA	10 (14.7)
Lesnoe mivirus	NA	NA	NA	NA	NA	1	1 (1.4)
Yanggou tick virus	NA	5	NA	NA	NA	NA	5 (7.3)
<i>Hepeviridae</i> sp.	NA	NA	NA	1	NA	NA	1 (1.4)
Gakugsa tick virus	NA	NA	NA	NA	2	NA	2 (2.9)
Nairoviridae sp. isolate L1	NA	NA	NA	NA	NA	1	1 (1.4)
Alxa tick phlebovirus	NA	NA	NA	1	NA	NA	1 (1.4)
Blacklegged tick	NA	NA	NA	NA	1	NA	1 (1.4)
phlebovirus							
Bole tick virus 1	NA	NA	11	NA	NA	NA	11 (16.1)
Changping tick virus 1	NA	2	NA	NA	NA	NA	2 (2.9)
Mukawa virus	NA	NA	NA	NA	NA	1	1 (1.4)
Onega tick phlebovirus	NA	NA	NA	NA	NA	1	1 (1.4)
Sara tick phlebovirus	NA	NA	NA	NA	NA	7	7 (10.2)
Taishun tick virus	NA	NA	NA	6	NA	NA	6 (8.8)
Bole H. asiaticum virus 1	NA	NA	NA	4	NA	NA	4 (5.8)
Bole tick virus 4	NA	NA	NA	5	NA	NA	5 (7.3)
Bunyavirales spp. isolate	NA	NA	NA	NA	NA	1	1 (1.4)
L1b							
Hubei toti–like virus 24	NA	3	NA	NA	NA	NA	3 (4.4)
I. scapularis-associated	NA	NA	NA	NA	NA	1	1 (1.4)
virus 1							
Xinjiang tick–associated	NA	2	NA	NA	NA	NA	2 (2.9)
	Virus Bole tick virus 3 Lesnoe mivirus Yanggou tick virus Hepeviridae sp. Gakugsa tick virus Nairoviridae sp. isolate L1 Alxa tick phlebovirus Blacklegged tick phlebovirus Bole tick virus 1 Changping tick virus 1 Mukawa virus Onega tick phlebovirus Sara tick phlebovirus Sara tick phlebovirus Sara tick phlebovirus Taishun tick virus Bole <i>H. asiaticum</i> virus 1 Bole tick virus 4 Bunyavirales spp. isolate L1b Hubei toti–like virus 24 <i>I. scapularis</i> –associated virus 1 Xinjiang tick–associated virus 1	VirusSingle, n = 2Bole tick virus 3NALesnoe mivirusNAYanggou tick virusNAYanggou tick virusNAHepeviridae sp.NAGakugsa tick virusNAMairoviridae sp. isolate L1NAAlxa tick phlebovirusNABlacklegged tickNABlacklegged tick virus 1NAChangping tick virus 1NAOnega tick phlebovirusNASara tick phlebovirusNABole tick virus 1NAOnega tick phlebovirusNABole tick virus 4NABole tick virus 4NABole tick virus 4NABuyavirales spp. isolateNAL1bHubei toti-like virus 24Hubei toti-like virus 24NAI. scapularis-associatedNAVirus 1Xinjiang tick-associatedNAXinjiang tick-associated	Dermacentor nuttalliVirusSingle, n = 2n = 15Bole tick virus 3NANALesnoe mivirusNANAYanggou tick virusNANAYanggou tick virusNANAYanggou tick virusNANAGakugsa tick virusNANAGakugsa tick virusNANAMairoviridae sp.Isolate L1NANANANABlacklegged tickNANABlacklegged tickNANAChangping tick virus 1NANAChangping tick virus 1NANAOnega tick phlebovirusNANASara tick phlebovirusNANABole tick virus 4NANABole tick virus 4NANABole tick virus 4NANAL1bHubei toti-like virus 24NAHubei toti-like virus 1NANAXinjiang tick-associatedNANAXinjiang tick-associatedNA2virus 1NANA	InvanionDermacentor nuttalliasiaVirusSingle, n = 2n = 15n = 11Bole tick virus 3NANANANALesnoe mivirusNANANANAYanggou tick virusNASNANAYanggou tick virusNANANAYanggou tick virusNANANAYanggou tick virusNANANAYanggou tick virusNANANAYanggou tick virusNANANAGakugsa tick virusNANANAAlaroviridae sp. isolate L1NANANAAlxa tick phlebovirusNANANABlacklegged tickNANANAphlebovirusNANANABole tick virus 1NANANAOnega tick phlebovirusNANANASara tick phlebovirusNANANABole tick virus 4NANANABole tick virus 4NANANABole tick virus 4NANANABole tick virus 4NANANABunyavirales spp. isolateNANANAL1bHubei toti-like virus 24NANANANANANANANAXinjiang tick-associatedNA2NAVirus 1NANA2NAVirus 1NANA2NA<	InvalorminaDermacentor nuttalliasiaticumVirusSingle, n = 2n = 15n = 11n = 15Bole tick virus 3NANANANA10Lesnoe mivirusNANANANANAYanggou tick virusNA5NANAYanggou tick virusNA5NANAYanggou tick virusNANANANAYanggou tick virus 1NANANANAAlxa tick phlebovirusNANANANABole tick virus 1NANANANAChangping tick virus 1NANANANAOnega tick phlebovirusNANANANASara tick phlebovirusNANANANABole tick virus 4NANANANABole tick virus 4NANANANABole tick virus 4NANANANAHubei toti-like virus 24NANANANAL1bVirus 1NANANAXinjiang tick-associatedNA<	Dermacentor nuttalliasiaticumpersuVirusSingle, n = 2n = 15n = 11n = 15n = 10Bole tick virus 3NANANANANANALesnoe mivirusNANANANANAYanggou tick virusNASNANANAYanggou tick virusNANANANANAHepeviridae sp.NANANANANAGakugsa tick virusNANANANANAAlxa tick phlebovirusNANANANANAAlxa tick phlebovirusNANANANANABole tick virus 1NANANANANABole tick virus 1NA2NANANAMukawa virusNANANANANAOnega tick phlebovirusNANANANAAlxa tick phlebovirusNANANANAAlxa tick phlebovirusNANANANAMukawa virusNANANANAOnega tick phlebovirusNANANANABole H. asiaticum virus 1NANANANABole tick virus 4NANANANABole tick virus 4NANANANABunyavirales spp. isolateNANANANALibHubei toti-like virus 24NANANAN	InvolutionDermacentor nuttalliasiaticumPooled, Single, n = 2Single, Pooled, n = 11Single, Pooled, n = 10Single, Pooled, n = 10Bole tick virus 3NANANANA10NANALesnoe mivirusNANANANANANANAYanggou tick virusNASNANANANANAYanggou tick virusNANANANANANAGakugsa tick virusNANANANANANAGakugsa tick virusNANANANANANAAlza tick phlebovirusNANANANANANABlacklegged tickNANANANANANAMukawa virusNANANANANANAOnega tick phlebovirusNANANANANANABole tick virus 1NANANANANANAOnega tick phlebovirusNANANANANANABole tick virus 1NANANANANANABole tick virus 1NANANANANANABole tick virus 4NANANANANANABole tick virus 4NANANANANANABole tick virus 4NANANANANANABuny

Appendix 1 Table 2. Prevalence of viruses with unknown health impact.

NA, not applicable.



Appendix 1 Figure 1. Schema of the screening approach.



Appendix 1 Figure 2. Maximum-likelihood consensus tree of the partial *Rickettsia secY*, *rplO*, and *rpmD* genes (1,442 nucleotides), constructed using Tamura-Nei model, gamma distributed with invariant sites (G+I) for 500 replications. Red diamond indicates *Rickettsia canadensis* sequence obtained in the study and indicated with sample identifier. *Rickettsia* strains are indicated by GenBank accession number, name, and isolate identifier.

mutS

uvrD



Appendix 1 Figure 3. Maximum-likelihood consensus tree of the *Rickettsia mutS* (2,649 nucleotides) and *uvrD* (1,080 nucleotides), constructed using Tamura-Nei model, gamma distributed with invariant sites (G+I) for 500 replications. Sequences obtained in the study are marked and indicated with sample identifiers. *Rickettsia* strains are indicated by GenBank accession number, name, and isolate identifier.



Appendix 1 Figure 4. Maximum-likelihood tree of the Alongshan virus and Yanggou tick virus NS5-like protein sequences (segment 1; 1,482 nucleotides), constructed using Tamura-Nei model, gamma distributed with invariant sites (G+I) for 500 replications. Sequences obtained in the study are marked (purple square and blue octagon) and indicated with sample identifiers. Virus strains are indicated by GenBank accession number, name and isolate identifier. Jingmen tick virus strain SY84 was included as an outgroup.



Appendix 1 Figure 5. Maximum-likelihood tree of the Alongshan virus and Yanggou tick virus VP1a-1b sequences (segment 2; 1,080 nucleotides), constructed using Tamura-Nei model, gamma distributed with invariant sites (G+I) for 500 replications. Sequences obtained in the study are marked (purple square and blue octagon) and indicated with sample identifiers. Virus strains are indicated by GenBank accession number, name and isolate identifier. Jingmen tick virus strain SY84 was included as an outgroup.



Appendix 1 Figure 6. Maximum-likelihood tree of the Alongshan virus NS3-like protein sequences (segment 3; 1,482 nucleotides), constructed using Tamura-Nei model, gamma distributed with invariant sites (G+I) for 500 replications. Sequence obtained in the study is marked (purple square) and indicated with sample identifiers. Virus strains are indicated by GenBank accession number, name and isolate identifier. Jingmen tick virus strain SY84 was included as an outgroup.



Appendix 1 Figure 7. Maximum-likelihood likelihood tree of the Alongshan virus and Yanggou tick virus VP2-3 sequences (segment 4; 1,080 nucleotides), constructed using Tamura-Nei model, gamma distributed with invariant sites (G+I) for 500 replications. Sequences obtained in the study are marked (purple square and blue octagon) and indicated with sample identifiers. Virus strains are indicated by GenBank accession number, name and isolate identifier. Jingmen tick virus strain SY84 was included as an outgroup.