Targeted Next-generation Sequencing for Biosurveillance in Morocco at African Lion 2026

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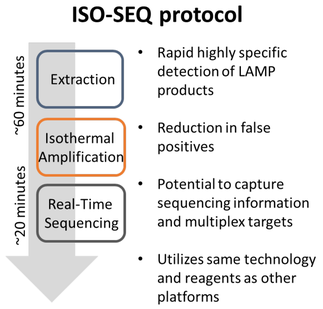
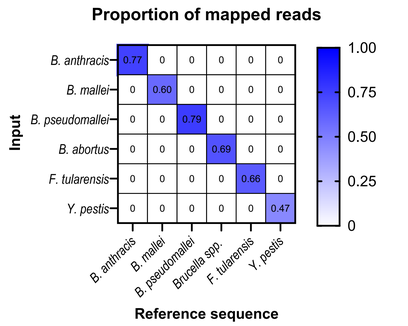
Introduction

African Lion (AL) is U.S. Africa Command's largest annual joint exercise, focused on enhancing warfighter capabilities with new technologies and strengthening military readiness and regional partnerships. At AL exercises, personnel from USAMRIID in partnership with GEIS conducted insect vector and veterinary biosurveillance (BSV) efforts of infectious disease pathogens that pose a risk to force health protection and demonstrated the ability to deploy targeted next generation sequencing (tNGS).

Methods

Samples were extracted using the Qiagen viral RNA kit or MagMAX Pathogen Nucleic Acid Isolation kit (ThermoFisher) after manual homogenization. At AL26, extracted samples were tested with multiplex loop-mediated isothermal amplification (LAMP) followed by next-generation sequencing (ISO-Seq). Assays targeting eight bacterial species (*Bacillus anthracis, Burkholderia mallei, Burkholderia pseudomallei, Brucella* spp.*, Francisella tularensis* FopA*, Yersinia pestis* 3a*, Borrelia* spp.*, and Rickettsia* spp.) and four viruses (Crimean-Congo Hemorrhagic Fever virus [CCHFV], West Nile Virus [WNV], Chikungunya virus [CHIKV], and Toscana virus [TOSV]) were used. Following LAMP, the amplified product sequencing library was prepared using the Oxford Nanopore Technologies (ONT) Rapid Barcoding Kit. Libraries were sequenced using the ONT MK1D and R10 flow cells. Correlative results were performed by PCR using the Biofire FilmArray and Biomeme Franklin.

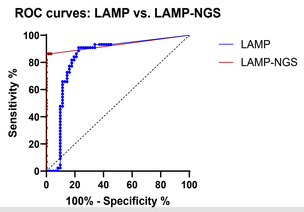




ISO-Seq workflow and diagnostic accuracy. The sensitivity and specificity of the LAMP-NGS (ISO-Seq) workflow is demonstrated by the proportion of mapped amplicon reads to target sequences in a multiplex assay panel (above), performance matrix in complex biological samples (below, left), and ROC curve analyses (below, right).

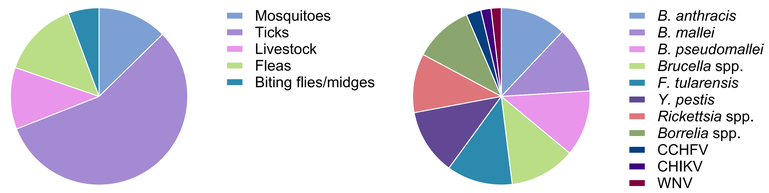
Mice exposed to *B. pseudomallei* (brain homogenate, N=53)

	PCR (+)	PCR (-)	
LAMP-NGS (+)	**19**	**0**	PPV = 100%
LAMP-NGS (-)	**3**	**31**	NPV = 91%
	Sensitivity = 86%	Specificity = 100%	

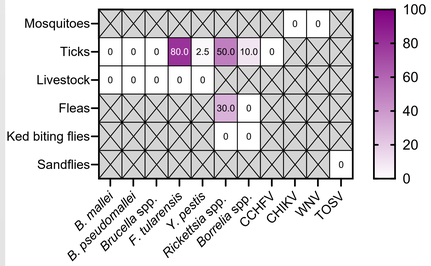


Results

A total of 483 tests were performed on 71 individual insect and animal samples. LAMP was performed on extracted nucleic acids in multiplex assays dependent on the vector/animal species tested. Ticks were tested for bacterial biothreats (*B. anthracis, B. pseudomallei, Brucella* spp.*, F. tularensis,* and *Y. pestis*), *Rickettsia* spp., *Borrelia* spp., and CCHFV. Mosquitoes were tested for WNV and CHIKV. Sandflies were tested for TOSV. Ked Biting Flies were tested for *Rickettsia* spp. and *Borrelia* spp. Fleas were tested for bacterial biothreats, *Rickettsia* spp. and *Borrelia* spp., and livestock blood was tested for bacterial biothreats.



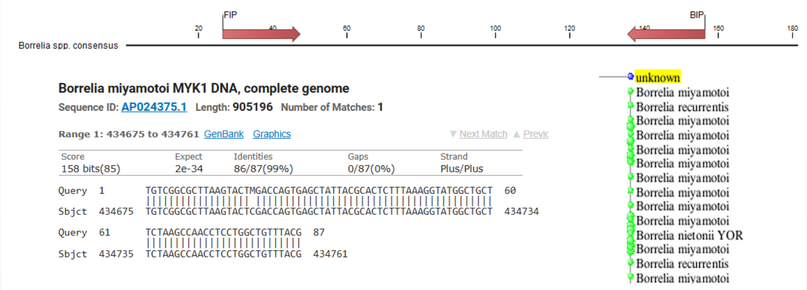
BSV sample species and assays performed. Whole insect vectors and livestock blood (N=71) were homogenized for tNGS (left). 483 individual LAMP assays were performed in multiplex panels dependent on pathogens relevant to each sample species.



Rickettsia spp. (representative sampling)			
Sample ID	**Vector**	**PCR**	**ISO-Seq**
AL26_039.03	Flea	ND	ND
AL26_039.04	Flea	ND	ND
AL26_039.05	Flea	Detected	Detected
AL26_042.01	Flea	ND	ND
AL26_042.02	Flea	Detected	Detected
AL26_042.03	Flea	Detected	Detected
AL26_042.04	Flea	ND	ND
AL26_043.01	Flea	ND	ND
AL26_043.02	Flea	ND	ND
AL26-25.01	Tick	ND	ND
AL26-25.02	Tick	ND	ND
AL26-26.01	Tick	ND	ND
AL26_31.01.L	Tick	Detected	ND
AL26_33.01.L	Tick	Detected	Detected
AL26_33.02.L	Tick	Detected	Detected
AL26_33.03.L	Tick	Detected	Detected
AL26_33.04.L	Tick	Detected	Detected
AL26.33.05.L	Tick	Detected	Detected
Total:		**26**	**23**

Incidence of sampling targets. tNGS identified *Rickettsia* spp. (23/52), *Borrelia* spp. (4/52), *F. tularensis* FopA (32/58), and *Y. pestis* 3a gene fragment (1/58). Further DNA sequence analysis and epidemiological data suggest that F. tularensis positives are more likely *Francisella*-like endosymbionts (FLEs), which share a significant amount of genetic similarity with *F. tularensis* and are highly prevalent in *Hyalomma* spp. ticks. Samples tested negative *for B. anthracis, B. mallei, B. pseudomallei, Brucella* spp., CCHFV, WNV, CHIKV, and TOSV. 26/50 samples (52%) were positive by PCR, while 23/52 (44%) were positive by ISO-Seq. An engorged *Hyalomma* tick is pictured, photo courtesy of MAJ Paul Lenhart, Ph.D.

Results (cont.)



Borrelia LAMP amplicon sequencing analysis. NCBI BLAST alignment of the inter-primer region of a consensus pan-Borrelia amplicon revealed highest-confidence matches to *Borrelia miyamotoi* (99-100% coverage). Phylogenetic analysis of the consensus amplicon sequence demonstrated clustering with majority *B. miyamotoi* reference sequences (excerpt of tree, right).

Conclusions

tNGS for BSV at African Lion identified the presence of 60 organisms in 71 insect vectors captured locally in Morocco. Further LAMP amplicon sequence analysis and epidemiological data corroborated the Rickettsia and Borrelia species findings in *Ctenocephalides felis* fleas and *Hyalomma* species ticks. However, assays designed for human pathogens *F. tularensis* most likely detected *Francisella*-like endosymbionts (FLEs). FLEs are common commensal organisms of *Hyalomma* tick species in North Africa and share up to 100% sequence homology with the *F. tularensis* FopA gene. Future assay design parameters for BSV must consider homology with commensal organisms of insects.

Biosurveillance efforts at African Lion allowed military personal direct interaction with tNGS, advancing pathogen detection and diagnostic capabilities. Continued participation at AL demonstrated USAMRIID tNGS protocols offer a readily deployable BSV tool for pathogen detection in forward deployed operations. The successful preparation, deployment, and expansion of tNGS at AL25 and 26 highlights the critical role of the partnership between USAMRIID and GEIS in zoonotic biosurveillance for force health protection and warfighter readiness. This partnership also allows developers the chance to field novel technologies in exercise environments to receive feedback for future advancement.

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