Serological Biosurveillance for Spillover of Henipaviruses and Filoviruses at Agricultural and Hunting Human-Animal Interfaces in Peninsular Malaysia

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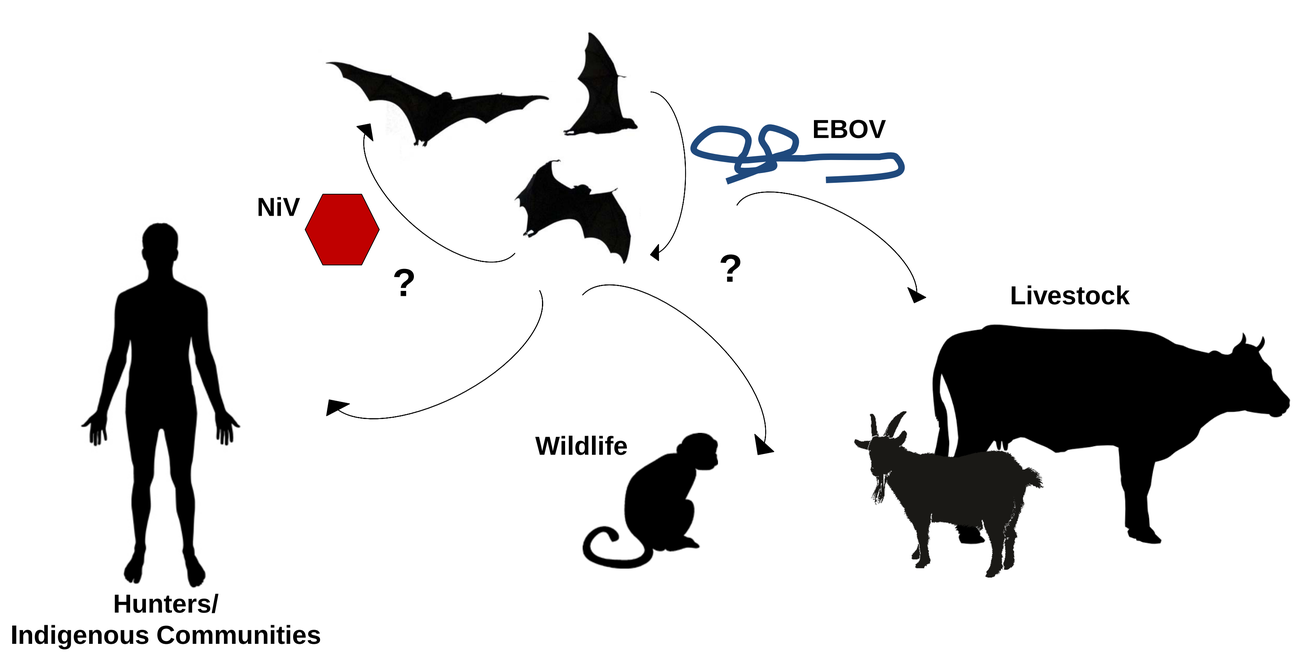
Background & Objectives

- **Filoviruses (e.g Ebola virus), and henipaviruses, (e.g Nipah virus), are 1) globally distributed, 2) naturally occur in bats, and 3) are global health and biosecurity threats.**
- **Hypothesis: Undetected transmission of henipaviruses and filoviruses among wildlife, livestock and people occurs in Malaysia.**
- **Objective 1: Characterize the distribution and detect spillover of henipaviruses and filoviruses within indigenous populations and on farms in Peninsular Malaysia in conjunction with the USAID Emerging Pandemic Threats: PREDICT project.**
- **Objective 2: Enhance capacity of government partner labs for serological surveillance of highly pathogenic zoonotic viruses.**
- **Partners: Govt. of Malaysia and academic institutes including Department of Wildlife and National Parks, National Public Health Laboratory, Department of Veterinary Services, Universiti Putra Malaysia, and University of Malaya.**

Figure 1. A serological method will be used to characterize the diversity of filoviruses and henipaviruses, some with zoonotic potential, in natural bat host populations and to investigate whether undetected spillover has occurred at high risk interfaces such as on farms or in indigenous communities who hunt wildlife.



Serological Method: Multiplex Immunoassay

Table 1. Filoviruses and henipaviruses soluble envelope glycoproteins used in this multiplex assay & conjugated Bio-Plex® magnetic beads.

	Virus	Abbreviation	Bio-Plex® bead
	Filoviruses		
1	Ebola virus	EBOV	#34
2	Sudan virus	SUDV	#77
3	Reston virus (monkey isolate)	RESTVm	#85
4	Reston virus (pig isolate)	RESTVp	#72
5	Taï forest virus	TAFV	#57
6	Bundibugyo virus	BDBV	#64
7	Marburg virus	MARV	#37
8	Ravn virus	RAVV	#49
10	Lloviu virus	LLOV	#80
	Henipaviruses		
11	Nipah virus	NiV	#43
12	Hendra virus	HeV	#46
13	Cedar virus	CedPV	#53
14	Kumasi virus	KumPV	#40
15	Mojiang virus	MojPV	#50

Viruses in this table represent all presently characterized *Ebolavirus*, *Marburgvirus*, and *Henipavirus* species.

Advantages of Luminex-based Immunoassays

- **Screen for multiple virus targets in a single well; less time & reagent use vs. ELISA;**
- **Small vol. of sera (5µl) required to screen for antigen-specific IgG using entire panel of virus proteins; ideal when limited sample vol. available (e.g. bats & rodents);**
- **Antigens expressed as native-like oligomers so conformationally-dependent virus-specific antibodies are detected;**
- **Greater dynamic range; analysis of positive and negative samples more certain.**

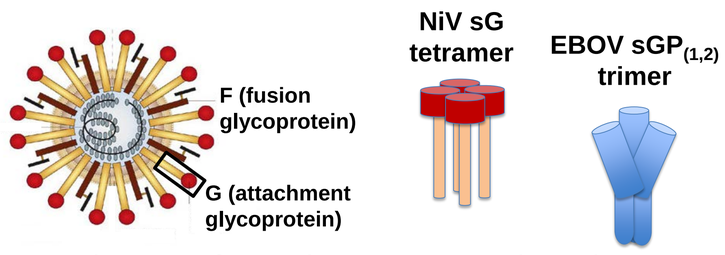
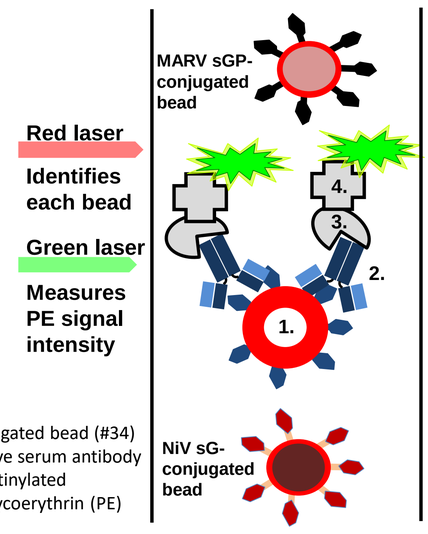


Figure 2. The ectodomains of recombinant virus envelope glycoproteins are produced in mammalian cell-culture systems and retain native-like oligomeric structure and glycosylation. Figure modified from Eaton BT, et al., *Nature Reviews Microbiology*. 2006;4:23-25.

Figure 3. The Bio-Rad Bio-Plex 200 HTF machine uses fluidics and optics to detect unique infrared and far-red bead signatures & measure fluorescent intensity.



1. EBOV sGP-conjugated bead (#34)
2. EBOV GP-reactive serum antibody
3. Protein A/G-biotinylated
4. Streptavidin-phycoerythrin (PE)

Achievements and Future Work

- **Bio-Rad Bio-Plex 200 HTF machines have been installed at DWNP National Wildlife Forensic Laboratory and the MOH National Public Health Laboratory.**
- **Staff from DWNP, NPHL, DVS, UPM, and UM have received theoretical and practical training to setup the multiplex assay with control sera samples and run the Bio-Plex 200 HTF machine.**
- **Preliminary data generated at partner labs using 1) archived bat sera from *Cynopterus, Pteropus, Rhinolophus, Penthetor, Murina, Hipposideros, Rousettus, Macroglossus and Tylonycteris* species and 2) archived *Macaca fascicularis* sera.**
- **A third Bio-Plex machine will be installed at UPM & staff training workshop delivered.**
- **Human sera screening at NPHL; confirmatory Western blot testing at UM; and human & animal farm sampling will begin in 2018.**

Previous biosurveillance application of multiplex immunoassay

Mean fluorescence intensity (MFI) values obtained from screening *Eonycteris spelaea* sera with filovirus sGPs. A dashed line indicates the cutoff value at 200 MFI.

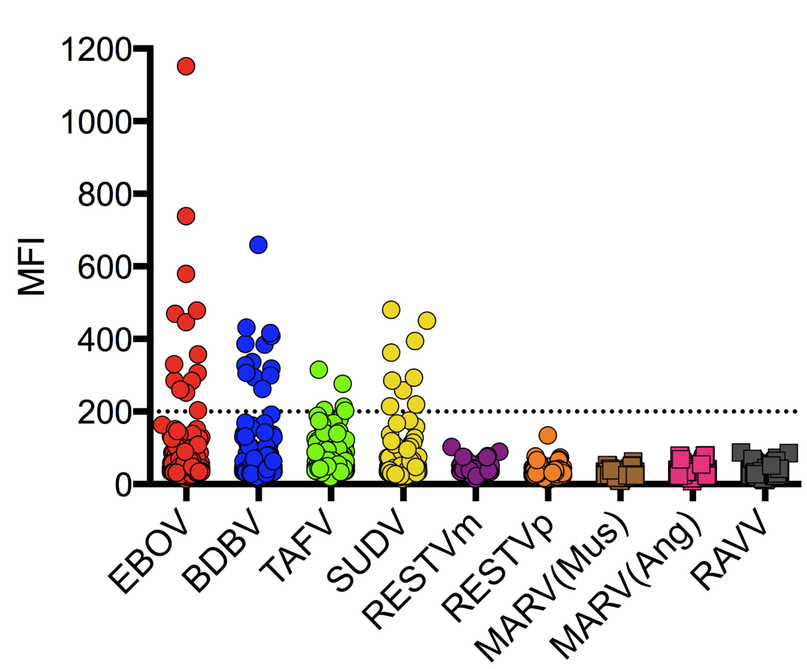


Figure from: Laing ED*, Mendenhall IH*, Linster M, *et al*. Serologic Evidence of Fruit Bat Exposure to Filoviruses, Singapore, 2011–2016. *Emerging Infectious Diseases*. 2018;24(1):122-126.

Acknowledgements

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