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Seroepidemiologic Survey of Crimean-Congo Hemorrhagic Fever Virus in Logging Communities, Myanmar

Appendix

Project Approvals

Study protocols were reviewed independently and ethical approval was provided by the Institutional Review Board (approval no. 889159–2) and Institutional Animal Care and Use Committee (approval no. 19520) at the University of California, Davis, the Ethics Review Committee of the Department of Medical Research (approval no. 012816), the Forest Department of the Ministry of Natural Resources and Environmental Conservation, the Livestock Breeding and Veterinary Department and the Myanmar Timber Enterprise.

Bead-Based Serologic Assay

Specific immunoglobulin G (IgG) reactivity against Crimean-Congo hemorrhagic fever virus (CCHFV) was detected by using a bead-based assay, MagPix (Luminex Corporation, https://www.luminexcorp.com), developed at the U.S. Army Medical Research Institute of Infectious Diseases. MagPix has demonstrated an enhanced sensitivity profile relative to conventional ELISA (*1*,*2*) and detailed methods have been described previously by Smith et al. (*3*). In brief, recombinant CCHFV nucleoprotein, produced in a baculovirus expression system and based on the IbAr10200 isolate (GenBank accession no. KY484036) as a reference strain, were conjugated to magnetic microspheres by using the xMAP Antibody Coupling Reagent Kit (Luminex Corporation) according to the manufacturer's instructions. Antigen coupled beads were combined with 1:100 diluted serum and analyzed on the MagPix instrument. Data were evaluated as signal to noise (S/N), with noise being the average median fluorescence intensity of each bead set in response to naive serum samples. We considered any sample with S/N >20 to be seropositive.

Assay Validation

In a comparative study evaluating the immune response to both CCHFV strain Kosova Hoti (GenBank accession nos. DQ133507, EU037902, EU044832) and strain Afg09–2990 (GenBank accession nos. HM452307, HM452306, HM452305) in experimentally infected Cynomolgus macaques (*Macaca fascicularis*), host antibody response was measured from 1–28 days post CCHFV inoculation by viruses derived from both IgG MagPix assay and neutralization assay. Virus-neutralization response was evaluated by using a virus-like particle (VLP) system with glycoproteins based on CCHFV strain IbAr 10200. We observed the emergence of neutralizing antibodies in serum samples by day 9 post infection for both groups, with broadly similar kinetics and endpoint titers between the Hoti and Afg09 infected groups. Mean fold increase (MFI) values averaging 5,737 corresponded with an 80% plaque reduction neutralization titer (PRNT₈₀) of 1:100 and MFI values averaging 10,243 corresponded with a PRNT₈₀ of 1:400. MagPix and PRNT₈₀ values both peaked at day 21 post inoculation.

Determination of Cutoff Value

To determine cutoff values, MFI and S/N were evaluated for a large multiregional serum set, 1,614 samples from Africa and 634 from Asia. Cutoff's were conservatively set at S/N of 20, far exceeding standard serologic assay cutoff algorithms that would have used 3 standard deviations above the mean of the negative controls.

PCR Assay for Bunyaviral Small, Medium, and Large Segments

Samples were processed for viral detection by using consensus PCR, which enables the universal amplification of sequences from viruses within a given family or genus, and the subsequent discernment of viral strains within. Total nucleic acid was extracted from whole blood by using Direct-zol RNA Miniprep Kits (Zymo Research, https://www.zymoresearch.com) according to the manufacturer's instructions. Total RNA was reverse transcribed into complementary DNA (cDNA) by using SuperScript III (Invitrogen, https://www.thermofisher.com) according to the manufacturer's instructions, and 3 assays were used for detection of bunyaviral small, medium, and large segments as described previously by Briese, et al. (*4*).

Statistical Analyses

To evaluate associations between human demographic and animal contact behaviors, all demographic factors, including age, sex, and livelihood, were first evaluated for associations with animal contact behaviors to assess potential confounding. Fisher exact tests were used to determine associations between CCHFV exposure and demographic as well as high-risk human– animal contact behaviors. Odds ratios were calculated by using a conditional maximum likelihood estimate method. For variables, in which 0-count cells were present, we calculated odds ratios by using an unconditional maximum likelihood estimate, Haldane-Anscombe correction. We considered p<0.05 statistically significant. Then we used multivariable logistic regression to assess the association between high-risk wild animal contact behaviors and other risk factors that were significant on bivariate analysis. Variables were included when they significantly improved model fit, based on the likelihood ratio test (p<0.1), while minimizing the Akaike information criterion. Overall model fit was assessed by using R version 3.6.1 (R Foundation for Statistical Computing, https://www.r-project.org).

References

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Human–Animal Contact Behavior Questionnaire

Participant ID:
1. Date of Interview
2. Where are you conducting this interview?
Village/City
District
Province/State
Latitude Longitude
Interviewer: Please collect GPS coordinates if administering using paper and pen.
Interview/Questionnaire Begins
3. How old are you?
If the exact age is unknown, enter the respondent's estimated age.
4. Where do you live?
Village/Town/City
District
Province/State

Interviewer: Probe for landmarks or nearest known site if area unknown. GPS coordinates to be identified and entered after completion of interview.

5.	How long have you lived there? Select one option.	<1 mo
		1 mo–1 y
		>1–5 y
		>5–10 y
		>10 y

6. How many other people live in the dwelling where you live? ______ Skip to question 9 if answer is 0.

7. How many in the dwelling are children less than 5 y old? _____

8. How many in the dwelling are male?

9.	ucture (that cannot be moved)? yes	Is the dwelling a permanent structure (that cannot be moved)?		yes
no	no		9.	no

	-	piped in water/water taps
De		covered well
10.	Do you get water from.	uncovered well/pond/river
	Select all that apply.	water truck/rainwater harvest
		other

11	11. Do you treat your drinking water?	yes
11. L		no
		boil

		501
12. If yes, how do you treat your water? Select all that apply.	If you have do you tract your water?	filter
	add chlorine or bleach	
	Select all that apply.	solar disinfection
		other

13.	Is your source for drinking water ever used by animals?	yes	
		no	
14. In your dwelling is there a dedicated location for human solid	yes		
	waste/excreta? (e.g., toilet, latrine, designated area)	no	

	What is the highest level of education you have completed?	primary school
15.	Select one option.	secondary school
		Finished 10 th standard

	college/university/professional
	none
	primary school

	What is the highest level of education that your mother completed?	secondary school
16.	Select one option.	Finished 10 th standard
		college/university/professional
		none

		1.	extraction of minerals, gas, oil, timber, coal
		2.	crop production
		3.	wildlife restaurant business
		4.	wild/exotic animal trade/market business
		5.	rancher/farmer animal production business
	Since this time last year what are the activities you	6.	meat processing, slaughterhouse, abattoir
17	have done to earn your livelihood?	7.	zoo/sanctuary animal health care
17.	Select all that apply.	8.	protected area worker
		9.	hunter/trapper/fisher
		10.	forager/gatherer/non-timber forest product collector
		11.	migrant laborer
		12.	nurse, doctor, traditional healer, community health worker
		13.	construction
		14.	other:

18. If more than one activity was selected, what is the activity on which you spent the most time since this time last year? * Write in the activity number from the above list. ______

	Which best describes your job position? Select one option.	manager (non-government)
		worker (non-government)
		manager (Government)
19.		Worker (Government)
		live and work at home independently (Skip to question 28)
		Professional (health worker, teacher)
		other:

20. Where do you work? (If different from where you live.) Village/Town/City ______ District _____

Province/State

Interviewer: Probe for landmarks or nearest known site if area unknown. GPS coordinates to be identified and entered after completion of interview.

Medical History Section

In this section, I'm going to ask you about any illness or sickness that is not known or recognized in the community, including by medical or treatment providers.

		clinic/health center
		hospital
21	Where do you usually get treatment for medical problems?	mobile clinic
21.	Select all that apply.	community health worker
		traditional healer
		dispensary or pharmacy

		fever with headache and severe fatigue or weakness (encephalitis)
		fever with bleeding or bruising not related to injury (hemorrhagic fever)
	In your lifetime, have you ever had	fever with cough and shortness of breath or difficulty breathing (SARI)
	an unusual illness with any of the following symptoms (READ ONLY SYMPTOMS) Select all that apply.	fever with muscle aches, cough, or sore throat (ILI)
22.		fever with diarrhea or vomiting
		fever with rash
		persistent rash or sores on skin
		no (Skip to question 33)
		yes but, none of these symptoms - describe

23.	Since this time last year, have you had any of these symptoms?	yes			
		no (Skip to question 29)			

		fever with headache and severe fatigue or weakness (encephalitis)
		fever with bleeding or bruising not related to injury (hemorrhagic fever)
		fever with cough and shortness of breath or difficulty breathing (SARI)
24	If yes, which ones?	fever with muscle aches, cough, or sore throat (ILI)
24.	Select all that apply.	fever with diarrhea or vomiting
		fever with rash
		persistent rash or sores on skin
		yes but, none of these symptoms - describe

		contact with sick people
		contact with wild animals
		contact with other animals
25	In your opinion, when you were sick, what caused this sickness?	bad food or water
25.	Select all that apply.	bad spirits/witchcraft
		wound or injury
		I don't know
		other:

26.	Since this time last year, have any of the people you lived with had any of	yes			
		No (skip to question 29)			

		fever with headache and severe fatigue or weakness (encephalitis)
		fever with bleeding or bruising not related to injury (hemorrhagic fever)
		fever with cough and shortness of breath or difficulty breathing (SARI)
27	If yes, which ones?	fever with muscle aches, cough, or sore throat (ILI)
27.	Select all that apply.	fever with diarrhea or vomiting
		fever with rash
		persistent rash or sores on skin
		yes but, none of these symptoms - describe

28.	Since this time last year, did anyone you lived with die from this illness?	yes
		no

Movement Section

In this section, I'm going to ask you about any travel you have done since this time last year.

29.	Have you traveled since this time last year?	
	If answer is no, skip to the next section.	yes
		no

30. Where have you traveled since this time last year? Anywhere else?

Interviewer: Probe for landmarks or nearest known site if area unknown. GPS coordinates to be identified and entered after completion of interview.

Collect up to 6 locations.

If there are more than six locations check here. Do not collect additional location information.

31.		work			
	Why have you traveled?	visit family			
	Select all that apply.	moved			
		religious reasons			

	holiday/vacation
	go to hospital/seek medical care
	go to market
	other:

Animal Contact Section

46.

have an open wound?

Interviewer: Do not read responses.

In this section, I'm going to ask you about the animals in your life.

If answered "no" under the "in your lifetime" column, then no answer is required under the "Since this time last year" column. Since this In your lifetime ... time last year .. yes yes 32. Has an animal lived as a pet in or near your dwelling? no no yes yes 33. Have you handled live animals? no no yes yes 34. Have you raised live animals? no no yes yes 35. Have you shared a water source with animals for washing? no no don't know don't know ves yes 36. Have you seen animal feces in or near food before you have eaten it? no no yes yes Have you eaten food after an animal has touched or damaged it? 37. no no For example, chew marks or scratches don't know don't know yes yes 38. Do any animals come inside the dwelling where you live? no no yes yes 39. Have you cooked or handled meat, organs or blood from a recently killed animal? no no yes yes 40. Have you eaten raw or undercooked meat or organs or blood? no no yes yes 41. Have you eaten an animal that you knew was not well /sick? no no don't know don't know Have you found a dead animal and collected it to eat or share? yes yes 42. Select all that apply. no no yes yes 43. Have you found a dead animal and collected it to sell it? no no yes yes 44. Have you been scratched or bitten by an animal? no no let someone else take over wash wound with soap and water The last time you were scratched, bitten or cut yourself while butchering or slaughtering, rinse wound with water 45. what did you do? bandage wound Select all that apply. visit doctor nothing - kept working never butcher or slaughter no

yes, but I don't know what they

yes, it can make you sick yes, it can poison you

are

Do you think there are any risks associated with slaughtering or butchering when you

	yes, it can infect you with a disease don't know										а				
											other				
47.	Have you slaughtered an animal? yes yes no no										es D				
48.	Have you hunted or trapped an animal?									es D					
		Circle all headings where "yes" was answere d in question s above.	pet (32)	handle d (33)	raise d (34)	feces in or near food (36)	in hous e (38)	cooked / handle d (39)	eaten raw/ under- cooke d (40)	eate n sick (41)	found dead (42/43)	scrato d/ bitt (44)	che ten	slaugh - tered (47)	hunted/ trapped (48)
		Elephant													
		rodents/ shrews													
	Ask which animals /mammals for each "yes" category.	bats													
		non- human primates													
		birds													
49.		carnivore s													
		ungulates													
		pangolins													
		Poultry/ot her fowl													
		goats/ sheep													
		swine													
		cattle/ buffalo													
		dogs													

	cats						

		coffee, tea, or cocoa plants
		fruit or nut trees
		oil tree plantation
		oil seed crops
		hardwood plantation
		dry grains
	Which arong are at this site?	sugar
50.	Select all that apply	vegetable or fruit crops
	Select all that apply.	pulses/legume
		fiber
		forages
		cover crops
		fallow fields
		rubber
		fruits or nuts

		Less than 1 y
51.		1-2 y
		2–5 у
	How long have the crops / plantations been growing here?	5–10 y
		11–20 y
		21–30 y
		Greater than 30 y

		Less than 1 y
		1-2 у
52	How frequently are crops / plantations harvosted?	2–5 у
52.	now nequently are crops / plantations narvested?	5–10 y
		11–20 y
		21–30 y

		rodents/shrews
		bats
		non-human primates
53.	What wild animals live in crops / plantations?	birds
		carnivores
		ungulates
		pangolins

		underground mining (by shafts or tunnels)
1		open surface mining
		hydraulic mining (high pressure water)
54.	Soloct and option	gathering, panning, or collecting
		oil well/gas field
		logging
		other

		coal			
		coltan			
		diamond or other gemstone			
		tin			
55	What product(s) are extracted?	gold/silver			
55.	Select one option.	lead			
		oil /gas			
		timber/plant			
		electricity			
		other (please specify)			

Box Do you have on the work stat? no 57. To the best of your knowledge, how many people work at this site? 10-100 58. How long have you worked at this site? 1 mo 58. How long have you worked at this site? 1 mo 58. How long have you worked at this site? 1 mo 59. Is there on-site food production? Yes 60. If yes, who pays for the cost of growing the food crops? The company 61. Is there meat available for consumption? Yes 62. If yes, where does the meat come from? Is med onsite 100-1000000000000000000000000000000000	50	De very live on the work site?				yes	
57. To the best of your knowledge, how many people work at this site?	56.	Do you live on the work site?				no	
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domestic/guardian animals					nds		
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			flooding				

-		
		chasing animals out
		nothing
		rodents/shrews
		bats
	What animals have you hunted since this time last year?	non-human primates
60	Soloct all that apply	birds
09.		carnivores
		ungulates
		civits
		pangolins
		snare
		bow
		hands

		nunuo
		gun
70	Since this time last year, what methods have you used to hunt/trap animals?	machete
70.	Select all that apply.	knife
		net
		cage
		trap
		other

	What is the purpose of your trapping or hunting? Select all that apply.		for consumption at home	for use of animal products at home	for sale for consumption	for sale alive at market	for sale of animal products	live trapping of nuisance animals for trans- location	culling of nuisance animals
74		rodents/shrews							
71.		bats							
		non-human primates							
		birds							
		carnivores							
		ungulates							
		pangolins							

Since this time last year, when you hunt or trap:

70	Are you expected to blood?	
12.	Are you exposed to blood?	no
72	Have you been controlled or hitten?	
13.	have you been scratched of bitten?	no

yes no

74.	Since this time last year, have you seen an outbreak of dead wild animals?
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75.	If yes, which wild animals? Select all that apply. (add species list)	rodents/shrews
		bats
		non-human primates
		birds
		carnivores
		ungulates
		pangolins

		touch it to see if it is still fresh	
		What do you do when you find an animal dead (not in a trap or shot by another hunter)? Select all that apply.	butcher in the forest
			smoke or cook in the forest
			take home to prepare
	76.		bury it
			report it to authorities
			take it to sell it
			nothing
			other
F			

How do you transport a dood onimal if you take it?	not wrapped	
77. Select all that apply. wrapped in leaves or other natural material		

		wrapped in plastic			
		on yourself / carry by hand in a bag			
		in a basket			
79	De veu use special protective equipment (e.g. shees, masks, gloves)?		yes		
70.	Do you use special protective equipment (e.g., shoes, masks, gloves)?		no		
		shoes/boots			
	If yes, which protective equipment? Select all that apply.	mask	mask		
79.		clothes	clothes		
		gloves			
		gown/apron			
		handling animals			
	When do you use protective equipment? Select all that apply.	slaughter			
80.		butcher			
		always on at work			
		other:			