Development And Validation Of Microarray-Based Serological Assay For Crimean-Congo Hemorrhagic Fever (CCHF) And Determination Of The Prevalence Of CCFV In Guinea

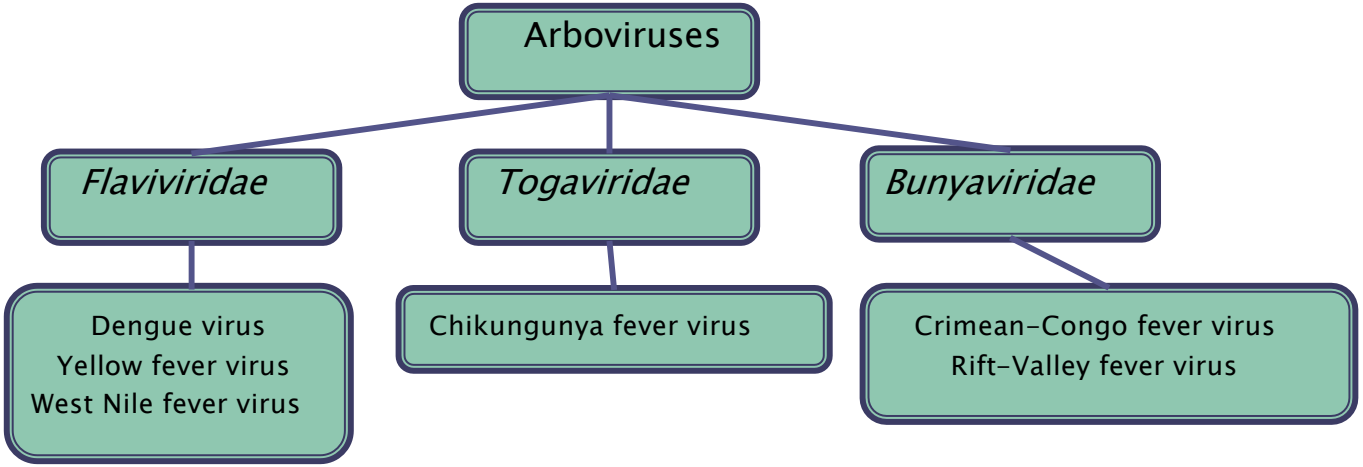
O. Stukolova

Central Research Institute of Epidemiology

Department of Molecular diagnostics and epidemiology

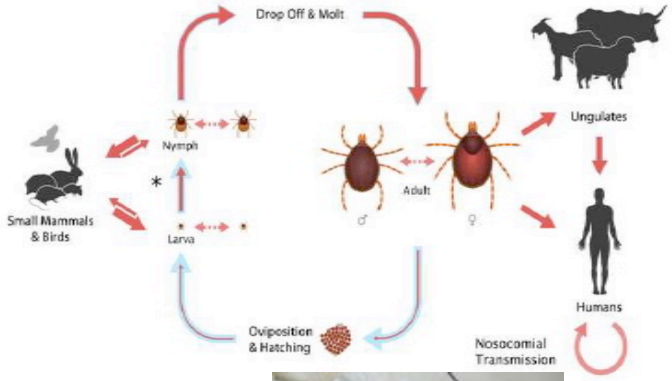
Moscow, Russia

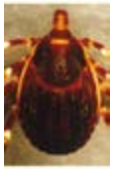
Project – Development and evaluation of the microarray based kit for diagnostic of arbovirus infections circulating in Russian Federation and Republic of Guinea or possible for importation to the countries

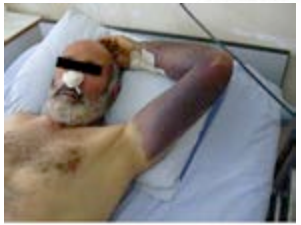


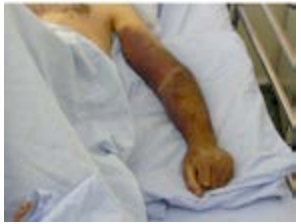
Crimean-Congo Hemorrhagic Fever

- First human disease cases were registered in 1944 in Crimea. The pathogen was determined in 1945. In 1956 the disease having similar symptoms were registered in Congo. The virological studies showed the identity of the pathogen with that has been found in Crimea
- Natural reservoirs of CCHFV – rodents, cattle, birds, wild mammalians, ticks
- Transmission is performed through tick bites; contact with blood, organs, body fluids of infected people or animals
- Clinic
  - Incubation period 1-10 days
  - First symptoms: fever, myalgia, dizziness, neck and back pain, neck muscle rigidity, headache, photophobia (duration 1-3 days)
  - Late symptoms: nasal bleeding, nausea, vomiting, diarrhea, abdominal pain, petechial rash, hepatomegaly, sudden mood swings, mental confusion (duration 4-10 days)
  - Symptoms of severe course: tachycardia, lymphadenopathy; ecchymosis, hemorrhages; hepatitis; kidney, liver or lung failure
  - If the course is severe, mortality rate is about 30%

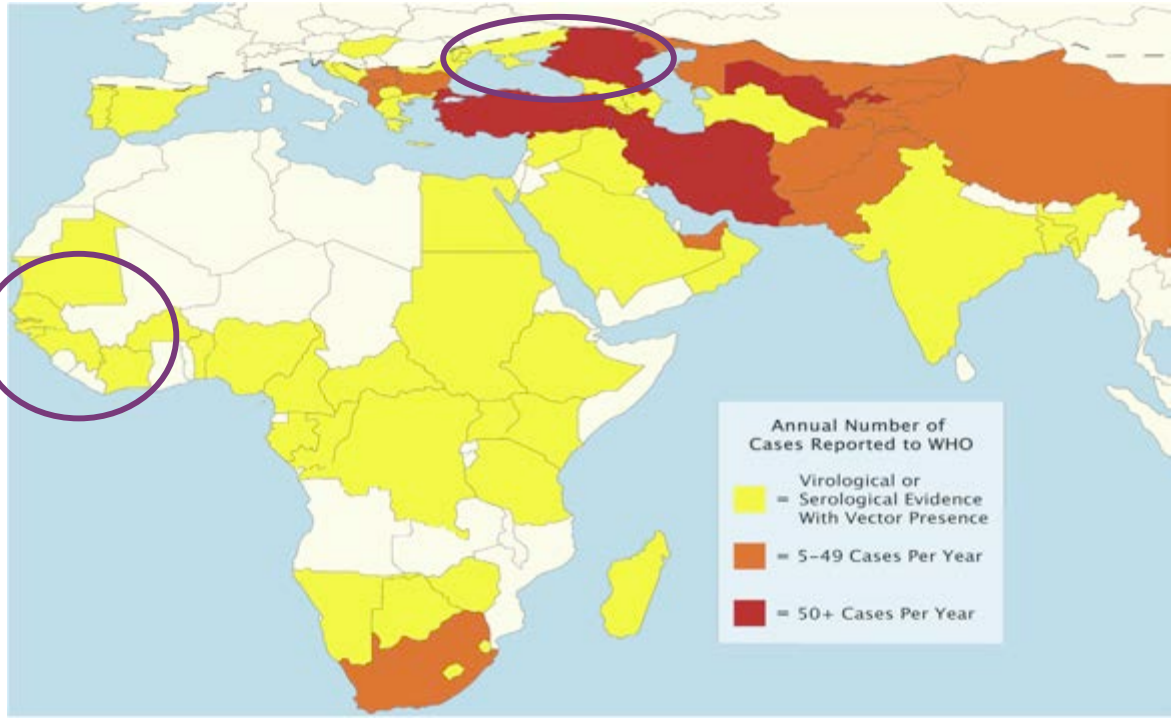








Prevalence of CCHF



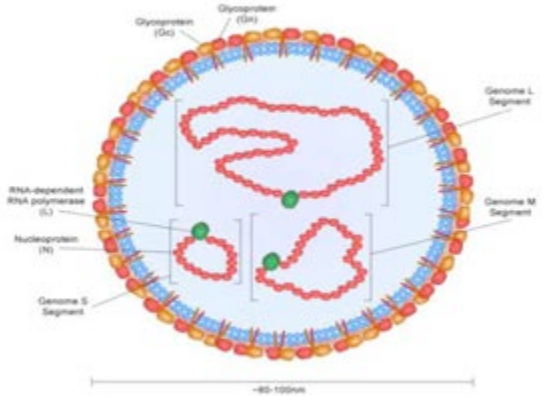
Meeting report: First International Conference on Crimean-Congo Hemorrhagic fever; A. Papa, F. Weber, R. Hewson et al; Antiviral Research 120 (2015) 57-65

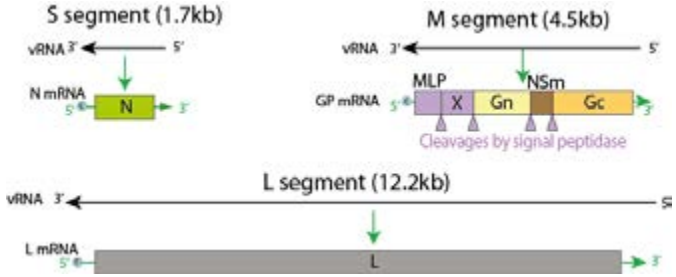
CCHF in Russia

Year	Astrakhan	Volgograd	Dagestan	Ingushetia	Kalmykia	Rostov	Stavropol	Total
2000	5	18	6	–	8	–	48	85
2001	11	9	10	–	3	5	21	59
2002	13	3	7	–	13	7	54	97
2003	9	3	3	–	23	9	30	77
2004	4	2	1	4	15	9	41	76
2005	47	6	3	–	38	16	38	138
2006	16	16	3	–	69	55	41	200
2007	20	30	2	1	64	53	63	234
2008	5	7	2	–	16	83	80	193
2009	6	2	1	–	17	27	66	119
2010	7	3	3	–	10	16	28	67
2011	10	2	2	–	11	48	26	99
2012	6	–	–	–	3	41	24	74
2013	1	6	–	–	–	38	32	80
2014	–	6	–	–	2	54	27	91
2015	1	3	–	–	9	79	49	138
2016	5	14	–	–	25	57	60	162
2017	2	4	–	–	14	28	19	79

CCHF diagnosis and virus structure

- Viral RNA detection – RT-PCR («Amplises® CCHFV-FL»)
- Antibody detection IgM (5-7 day) and IgG (2-3 weeks):
- Antigens for ELIS:
- Infected cell lysate – possible over diagnostics due to high cross-reactivity
- Recombinant protein – possible under diagnostics due to difficulties in expression

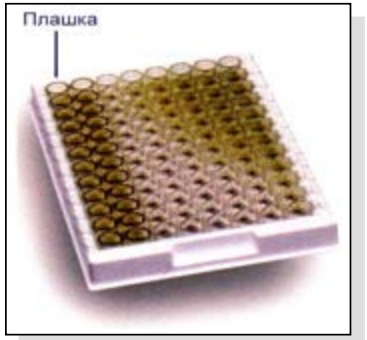




S segment – Nucleoprotin (NP)
M segment – Glicoproteins
L segment – RNA-dependent RNA polymerase

Protein Microarray

- one-step analysis for multiple markers
- very high informatively of test
- possibility to produce screening and confirmation tests in the same time



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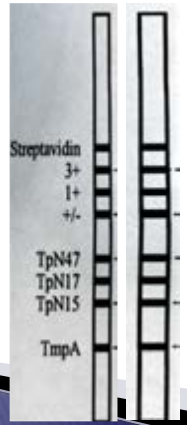

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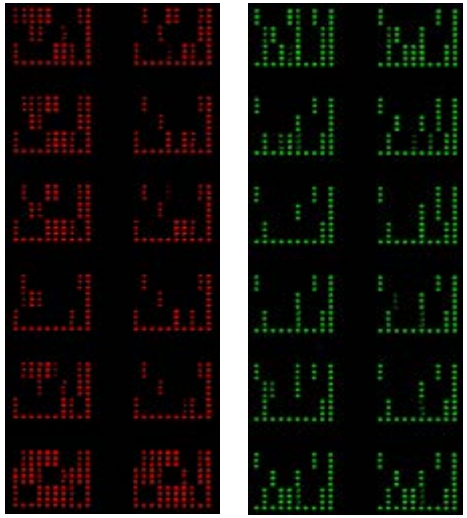
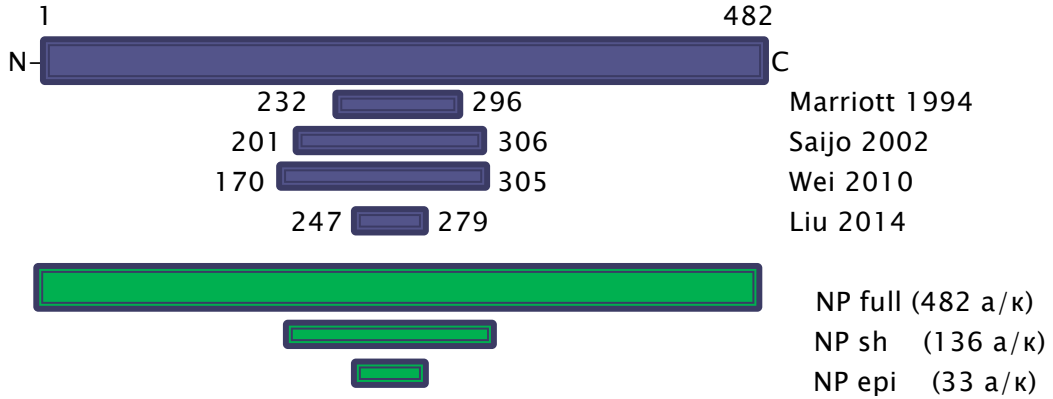
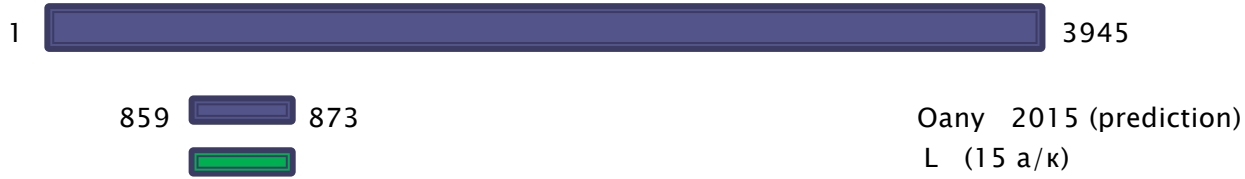


As sensitive as ELISA, as specific as blot

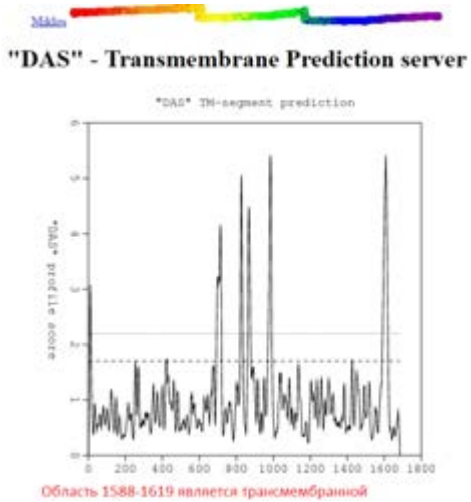
S segment – nucleoprotein (NP), antigenic regions



L segment – RNA-dependent RNA polymerase



M segment – Surface glycoproteins antigenic rigions



1443 1566 Ahmed

7

1172 1190 1451 1469 1613 1631 Goedhals 2014

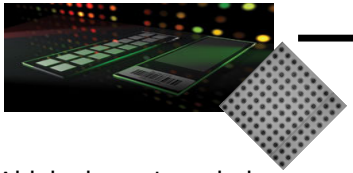
1181 1199 1622 1640

GpN (270 а/к)
GpC (322 а/к)

GP1 (19 а/к)
GP2 (19 а/к)

Manufacturing of the microarrays

Spotting of recombinant proteins and control solutions in triplicates



Aldehyde-activated glass-slides (VALS-25, Cell)
12 arrays on each slide

Procedure of analysis using the microarray based kit

Add sample + dilution 1 to 9 (5мин)

Incubation (30min)

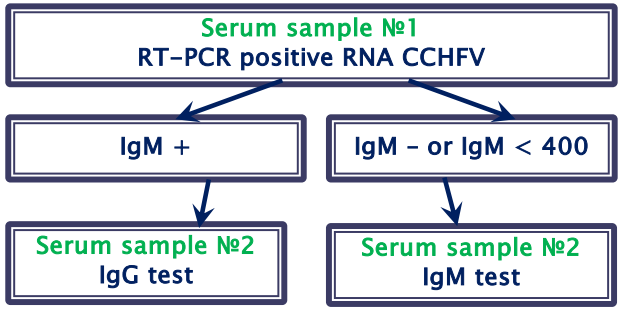
Washing (5мин)

Add conjugate mixture (Cy3-anti IgM + Cy5-anti IgG, incubation(30 min)

Washing (5мин)

Scanning, quantitation (15мин)

Serum cohort for microarray development



- **40 serum samples** collected in dynamics from 20 patients with CCHF on the endemic territories of Russia (Stavropol, Rostov). **Serum samples №1** were collected at day 4–7 after first symptoms onset, **Serum samples №2** were collected at day > 14 after first symptoms onset

- **20/20 Serum samples №1** were positive in RT-PCR «Amplisense CCHF-FL»

Recombinant proteins pre-tested & included in microarray for CCHF diagnostics:

- CCFV _ NP sh (AA 170–305 of nucleoprotein)
- CCFV _ GP 1 (AA 1451–1465 of surface glycoprotein G1)
- CCFV _ GP N (AA 1172–1443 of surface glycoprotein G1)
- CCFV _ GP C (AK 1246–1566 ofsurface glycoprotein G1)
- CFV _ L (AA 859–873 of protein L)

Interpretation rules were estimated using 200 serum samples of healthy controls from not endemic territory of Russia:

- IgG/IgM to one of G1 or L fragment – negative result
- IgG/IgM to two of G1 fragments – equivocal result
- IgG/IgM to NP and/or one G1 and L fragments together –positive result

Thus the specificity of microarray was determined to be 98%

Concentrations of anti-CCFV- specific IgM and IgG were interpolated from the human IgM and human IgG standard curves using ImStar software. **Specific IgM and IgG levels were considered significant if they exceeded 5 ug/ml**

IgM immune response in CCHF

Serum samples №1

		microarray CCHF			
		pos	neg	equivocal	total
ELISA Векто-Нил IgM	pos	10	1	3	14
	neg	0	6	0	6
	total	10	7	3	20

50%

Serum samples №2

		microarray CCHF			
		pos	neg	equivocal	total
ELISA Векто-Нил IgM	pos	18	0	1	19
	neg	0	1	0	1
	total	18	1	1	20

90%

70%

95%

	ELISA Векто-Нил IgM	CCFV_GP1	CCFV_L 1	CCFV_NP sh	CCFV_GP C	CCFV_GP N
1/1	neg	0.2	0.2	0	0	0
1/2	pos	0.1	0.1	4.3	0	0
9/1	pos	0.1	0.1	0	0	0
9/2	pos	0.1	0.1	0	0	0
10/1	pos	0.1	0.1	4.2	3.7	0.2
10/2	pos	1.4	1.9	4.3	36	1.5
14/1	pos	0	0	4.9	1.2	0
14/2	pos	0	0	8.1	0.2	0

	IgM to CCFV_GP 1	IgM to CCFV_L	IgM to CCFV_NP sh	IgM to CCFV_GP C	IgM to CCFV_GP N	no IgM
Serum samples №1	2/20	3/20	10/20	2/20	1/20	7/20
Serum samples №2	4/20	3/20	18/20	3/20	0/20	1/20

	IgM to CCFV_ NP sh	IgM к CCFV_ NP sh without IgM to other CCFV antigens	IgM to other CCFV antigens without IgM to CCFV_ NP sh
Serum samples №1	10/20	8/20	1/20
Serum samples №2	19/20	13/20	–

IgG immune response in CCHF

Serum samples №1

		microarray CCHF			
		pos	neg	equivocal	total
ELISA Векто-Нил IgG	pos	0	0	0	0
	neg	9	8	3	20
	total	9	8	3	20

45%

Serum samples №2

		microarray CCHF			
		pos	neg	equivocal	total
ELISA Векто-Нил IgG	pos	7	0	0	7
	neg	8	4	1	13
	total	15	4	1	20

75%

0%

	ELISA Векто-Нил IgG	CCFV_GP1	CCFV_L	CCFV_NP sh	CCFV_GP C	CCFV_GP N
3/2	IgG neg	8.7	12.9	4.9	0.2	2.5
4/2	IgG neg	0.5	0.7	13.1	2.5	2.5
8/2	IgG neg	0.3	0.1	26.1	0.2	0.2
15/2	IgG neg	2.5	3.9	13	30	0.1

35%

	IgG to CCFV_GP 1	IgG to CCFV_L	IgG to CCFV_NP sh	IgG to CCFV_GP C	IgG to CCFV_GP N	no IgG
Serum samples №1	2/20	3/20	9/20	1/20	3/20	8/20
Serum samples №2	1/20	1/20	15/20	2/20	2/20	4/20

	IgG to CCFV_ NP sh	IgG к CCFV_ NP sh without IgG to other CCFV antigens	IgG to other CCFV antigens without IgG to CCFV_ NP sh
Serum samples №1	9/20	6/20	3/20
Serum samples №2	15/20	12/20	1/20

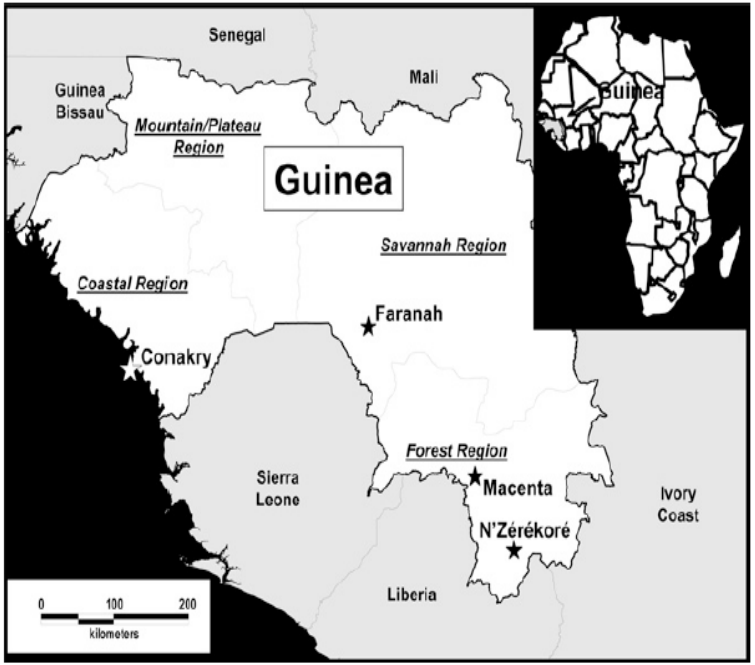
Conclusion 1

		CCFV_GP1	CCFV_L	CCFV_NP sh	CCFV_GP C	CCFV_GP N	CCFV_GP1	CCFV_L	CCFV_NP sh	CCFV_GP C	CCFV_GP N
1/1	IgM neg	0.2	0.2	0	0	0	0.2	0.3	1.0	0.5	0.2
1/2	IgM pos	0.1	0.1	4.3	0	0	0.4	1.1	5.8	0.5	0.2
9/1	IgM pos	0.1	0.1	0	0	0	2.1	1.5	10.2	0.3	1.1
9/2	IgG pos	0.1	0.1	0	0	0	0.2	1.3	50.3	0.2	0.2
10/1	IgM pos	0.1	0.1	4.2	3.7	0.2	0.7	1.1	10.2	1.1	0.4
10/2	IgG pos	1.4	1.9	4.3	36	1.5	2.1	3.0	107.7	2.5	1.2
14/1	IgM pos	0	0	4.9	1.2	0	0.2	0.3	0.2	1.1	0.5
14/2	IgM pos	0	0	8.1	0.2	0	0	0.1	0.2	0	0

- **Most important antigen of CCFV is NP** (IgM detected in 10/20 & 18/20 samples №1 & 2 respectively, IgG – in 9/20 & 15/20)
- Additional proteins helps to increase accuracy of diagnostics
- **Complex& separate detection of IgM and IgG allows to achieve the sensitivity of ELISA-test with high specificity rate**

	microarray	ELISA
Diagnosis after IgM detection in serum sample №1	10/20	14/20
Diagnosis after IgM&IgG detection in serum sample №1	17/20	14/20
Diagnosis after IgM detection in serum sample №1&2	18/20	19/20
Diagnosis after IgM&IgG detection in serum sample №1&2	20/20	20/20

CCHF in West Africa



- High prevalence of CCFV RNA in ticks in all countries of region
- Prevalence of CCHFV-specific IgG antibodies in the cattle samples was 66% (706/1,075; 95% confidence interval [CI]: 63–69%) in Mali
- Prevalence of CCHFV-specific IgG antibodies in the cattle samples was ~ 30% in Guinea
- From February to August 2003, 38 persons were infected with Crimean-Congo hemorrhagic fever (CCHF) virus in Mauritania
- IgM and IgG CCHFV seroprevalence rates in samples submitted from suspected yellow fever cases in Mali, 2009–2013: IgM+ 18/376 (4.8%), IgG+ 21/376 (5.5%), IgM/IgG + 0%
- Results of the IgG ELISA in humans in Borno State, Nigeria were IgG+ in 17/297 (5.7%)
- From 1978 to 1985 9 CCHF stains were isolated from Ixodidae ticks in Guinea

Am. J. Trop. Med. Hyg., 83(2), 2010, pp. 388–394
doi:10.4269/ajtmh.2010.09-0688

Determination of the prevalence of CCFV in Guinea

- **200 serum samples** were collected from healthy control group of miner workers, drivers and miner office workers in the Kindia region of Republic of Guinea during medical checkups

- **200/200 Serum samples** were negative in RT-PCR «Amplisense CCHF-FL»

№№	CCFV_Gp1	CCFV_L	CCFV_GpN	CCFV_GpC	CCFV_NP sh	CCVF IgG	CCVF IgM
6235	3,5	3,7	2,0	3,5	6,8	pos	equivocal
6326	0,0	0,1	11,9	14,8	0,1	equivocal	neg
6374	0,0	0,3	28,6	54,1	0,1	equivocal	neg
6931	17,4	17,6	1,3	3,3	14,5	pos	neg

IgM to CCFV antigens were found in 2 serum samples (1%)
IgG to CCFV antigens were found in 14 serum samples (7%)

№№	CCFV_Gp1	CCFV_L	CCFV_GpN	CCFV_GpC	CCFV_NP sh	CCVF IgG	CCVF IgM
5958	9,1	10,8	0,5	0,2	4,2	equivocal	neg
5981	0,4	0,6	0,3	0,2	32,8	pos	neg
6045	0,4	0,6	0,3	0,2	32,8	pos	neg
6111	0,8	1,4	6,1	9,5	0,4	equivocal	neg
6116	40,8	36,5	13,0	19,4	15,0	pos	neg
6228	1,3	2,2	0,2	0,1	1,7	pos	neg
6331	11,6	11,2	1,2	5,7	8,6	pos	neg
6358	4,2	7,9	0,1	0,2	7,5	pos	neg
6371	0,0	0,3	6,4	7,3	0,1	equivocal	neg
6400	0,2	0,2	0,3	0,5	6,6	pos	neg
6405	0,3	0,4	10,3	11,9	0,4	equivocal	neg
6409	13,2	14,4	4,7	8,4	5,4	pos	neg
6410	0,5	1,1	101,1	113,5	0,3	equivocal	neg
6465	0,3	0,5	0,3	0,1	16,9	pos	neg
7111	0,6	1,9	0,8	0,3	15,9	pos	neg
6915	1,0	2,3	2,3	3,1	5,5	pos	neg
6937	6,5	7,0	21,6	19,5	6,0	pos	neg
6940	11,7	12,4	0,7	1,0	14,5	pos	neg
6941	0,6	0,8	0,1	0,0	6,3	pos	neg

Conclusion

- Most important antigen of CCFV is NP (IgM were detected in 10/20 & 18/20 samples №1 & 2 from patients with RT-PCR confirmed CCFV infection respectively, IgG – in 9/20 & 15/20)
- We showed that developed microarray is highly sensitive and specific diagnostic mean
- Approximately 8% of the study population in Guinea has history of CCFV. This data consistent with data obtained for neighborhood countries
- As far the study is the first case of CCFV-prevalence determination in humans in Guinea.

Future investigations

- Determination of the prevalence of CCHFV-specific antibodies in different groups of healthy population (occupation, region diversity) in Guinea
- Determination of anti-CCFV IgM and IgG rates in samples submitted from patients with fever from different regions of Guinea
- Determination of the prevalence of CCHFV-specific antibodies in wild and domestic animals

Thank you for your attention!

olga.vasilyeva@cmd.su

Central Research Institute of Epidemiology, Department of Molecular diagnostics and epidemiology, Moscow, Russia
I. Goptar, L. Karan, A. Sudina, M. Polugalova, G. Shipulin

Stavropol anti-plague Institute, Stavropol anti-plague Institute, Stavropol, Russia
E. Kotenev, A. Kulichenko

Institute of Applied Biology in Guinea, Kindia, Guinea
M. Y. Boiro