RVF Diagnostic tools: surveillance, outbreak, and return to trade

Cairo 28/01/09

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of Nuclear Techniques in Food and Agriculture

Focus

- review of the available diagnostics for
  - surveillance,
  - during an outbreak, and
  - post-outbreak
- identify the gaps with current diagnostics
- discuss the most important tests in the pipeline.

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RVF: Foreword

- Rift Valley Fever Virus
  - Is a zoonotic agent → Hazard!
  - Can occur sub clinical
  - Has a broad host range in mammals
  - Has a broad vector range in mosquitoes + ticks
  - Might survive in mosquito eggs
  - Can be transmitted by ‘injection’ or close contact
  - Clinically recognized at sero-prevalence > 15%

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RVF Diagnostics: Caveat

- As there is no vaccination for humans
  - Reduce the risks!
    - Special caution when doing PM's
    - Handling of lab specimen (let serum clot....)
    - Reduce pipetting and dilution steps
    - Wash plates and equipment with caution
    - Work with safety measures

RVF serology: Facts

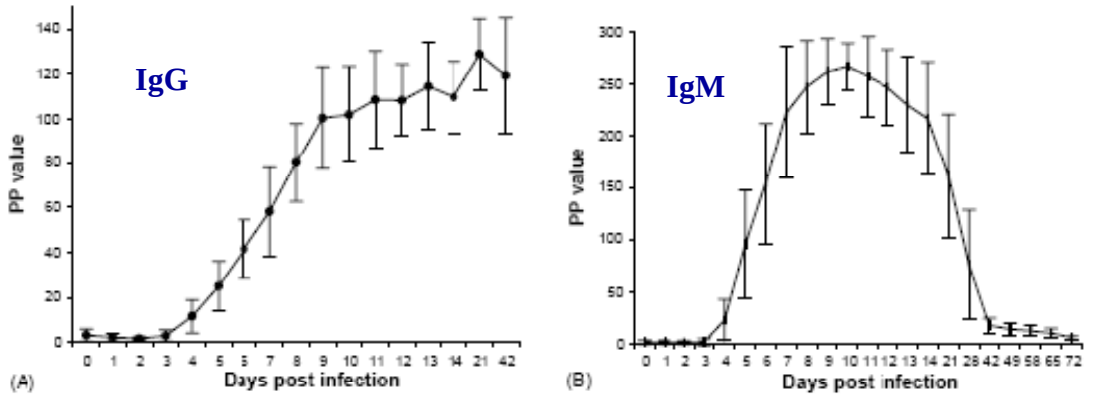

Fig. 1. Mean ± 1 S.D. IgG (A) and IgM (B) responses in sheep ($n = 8$) infected with wild type AR 20368 strain of Rift Valley fever virus.

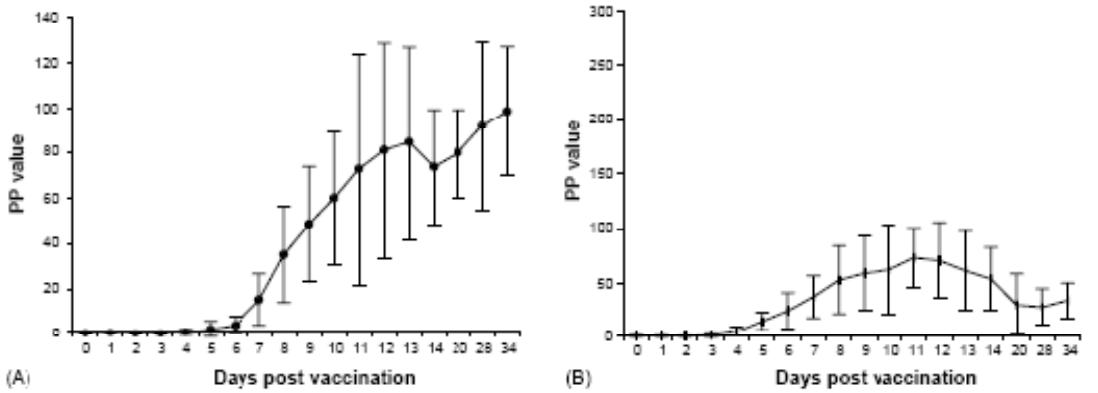

Fig. 3. Mean ± 1 S.D. IgG (A) and IgM (B) responses in sheep ($n = 10$) vaccinated with live-attenuated Smithburn strain of Rift Valley fever virus.

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Paweska , J Virol Methods. 2003

RVF virology: Facts

Virus distribution

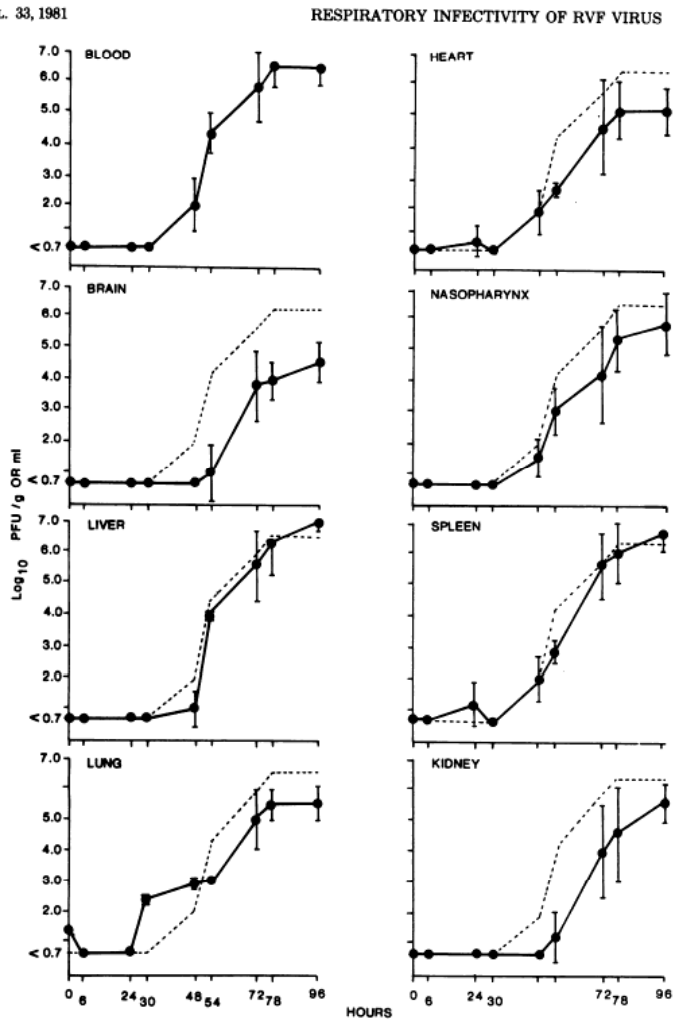

FIG. 1. *Virus concentrations in mice (n = 4) after exposure to 3.1 $\log_{10}$ PFU of RVFV strain ZH-501 via the respiratory route. Tissues from each mouse were assayed separately, in duplicate; each point represents the geometric mean, and the vertical bars indicate ± one standard error. Viremia, represented by broken lines, is superimposed on the plots for each tissue.*

Infect Immun. 1981, 33(3): J L Brown, et al. Respiratory infectivity of a recently isolated Egyptian strain of Rift Valley fever virus.

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RVF Virus persistence

- Data from the outbreak in Egypt in 1977 show 10^{10} RNA copies/mL of serum in sheep and 10^{8} copies/mL in cattle and humans
- Pid 9 the calf was no longer viremic, and RVF virus was isolated only from the brain....

RVF infectivity spectrum

Infectivity due to wild type virus:

Mortality ~ 100%	Severe illness: Abortion, mortality	Severe illness: Viremia, abortion	Infection: Viremia	Refractive to infection
Lambs	Sheep	Monkeys	Horses	Guinea pigs
Calves	Cattle	Camels	Cats	Rabbits
Kids	Goats	Rats	Dogs	Pigs
Puppies	Water buffalo	Gray squirrels	Monkeys	Hedgehogs
Kittens	Humans	Antelopes	Rock rat	Tortoises
White mice				Frogs
Hamsters				Chickens
Field mice				Canaries
Door mice				Pigeons
Field voles				Parakeets

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Experimental infections are less severe!

www.vet.uga.edu

RVF Diagnostics

Fitness for purpose

- Surveillance / prevalence
  - Domestic animals (sheep, goat, cattle, camel..)
  - Sentinel animals (sheep)
  - Wildlife
  - Vaccination
- Outbreak / incidence
  - Sero-diagnosis (IgM)
  - Virus diagnosis (ELISA, molecular)
- Post outbreak / trade

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Available test reagents

- Antigens
  - Cell culture derived
  - Mouse brain derived
  - Expression antigens
    - NSP
    - RVFV Nss protein
- Antibodies
  - Polyclonal (sheep, mouse)
  - MoAb

Available tests

- Commercially:
  - BDSL.....
    - inhibition ELISA for detecting IgG (in all species).
    - capture ELISA for IgM (in specified species, bov. capr. ovi)
    - indirect ELISA for IgG (anti-species conjugate)
    - Sandwich ELISA for IgG (in specified species, bov. capr. ovi)
- Through research links
  - CDC
    - RVF IgG ELISA; TC MP-12 based + of mock- antigen (Niklassonet al. J Clin Microbiol. 1983;17:1026–31.


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Laboratory tests

- Virus neutralisation test
- Virus culture
- Agar gel tests

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Specific tests: IgG Sandwich ELISA (SA)

- **Coating:** 100 µl/well of mouse anti-RVFV serum diluted 1:5000 in PBS on MaxiSorp, ELISA plates +4^{O}C overnight.
- Wash three times with 0.1% Tween-20 in PBS;
- **Blocking:** with 200µl/well 10% skim milk in PBS for 1 h
- Wash three times with 0.1% Tween-20 in PBS;
- **2nd coating**: 100 µl/well of RVFV and control antigens, diluted 1:400 in 2% skim milk in PBS ; incubation for 1 h at 37^{O}C,
- Wash three times with 0.1% Tween-20 in PBS;
- **Samples:** 100 µl diluted 1:400 in diluent buffer; incubation at 37^{O}C for 1 h,
- Wash three times with 0.1% Tween-20 in PBS;
- **Conjugate:** 100µl/well anti-sheep IgG HRPO-conjugate 1:8000 ; incubation at 37^{O}C
- Wash six times with 0.1% Tween-20 in PBS;
- **Substrate:** ABTS peroxidase ; incubated in the dark for 30 minutes at room temperature
- **Stop reagent,** 1 % SDS,
- Read optical densities (OD) at 405 nm

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Specific tests: IgG Sandwich ELISA (US)

- **Coating:** 100 µl/well of mouse anti-RVFV serum diluted 1:160 in coating buffer (including BSA) on Dynatech ELISA plates; incubate at 37^{O}C , 1 h.
- Wash three times with 0.05% Tween-20 in PBS; ;
- **2nd coating**: 100 µl/well of RVFV or Vero cell antigens, diluted 1:100 in 0,5% BSA in PBS ; incubation for 1 h at 37^{O}C,
- Wash three times with 0.05% Tween-20 in PBS;
- **Samples:** 100 µl diluted 1:40 in ELISA buffer + 1% mouse serum were added; incubation at 37^{O}C for 1 h,
- Wash four times with 0.05% Tween-20 in PBS;
- **Conjugate:** 100µl/well alkaline phosphatase-conjugated swine-
- anti-human IgG, diluted 1:200 in ELISA buffer; incubation at 37^{O}C
- Wash four times with 0.05% Tween-20 in PBS;
- **Substrate:** 100µl/well PNP (p-nitrophenyl-phosphate in diethanolamine)
- Buffer; incubated in the dark for 30 minutes at room temperature
- Read optical densities (OD) at 405 nm

There are several modifications of this test !

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Specific tests: *IgM capture ELISA*

- **Coating:** 100 µl/well of rabbit anti-sheep IgM diluted 1:500 in PBS onto MaxiSorp ELISA plates, at +4^{O}C overnight.
- Wash three times with 0.1% Tween-20 in PBS;
- **Blocking:** with 200µl/well 10% skim milk in PBS for 1 h
- Wash three times with 0.1% Tween-20 in PBS;
- **Samples:**, Add 100 µl samples diluted 1:400 in diluent buffer to wells in rows A-D: 1-12 and the corresponding wells in rows E-H: 1-12, incubate 37^{O}C for 1 h.
- Wash three times with 0.1% Tween-20 in PBS;
- **Capture Antigen**: Add 100 µl of RVFV antigen, diluted 1:200 to rows A-D: 1-12, and control antigen to rows E-H: 1-12 respectively. incubate 37^{O}C for 1 h
- Wash six times with 0.1% Tween-20 in PBS;
- **Capture antibody**: Add 100 µl/well of mouse anti-RVFV serum diluted 1:1000, incubate 37^{O}C for 1 h.
- Wash six times with 0.1% Tween-20 in PBS;
- Conjugate: Add 100µl/well anti-mouse HRPO diluted 1:8000 incubate 37^{O}C for 1 h.
- Wash six times with 0.1% Tween-20 in PBS;
- **Substrate:** ABTS peroxidase; incubated in the dark for 30 minutes at room temperature
- **Stop reagent,** 1 % SDS,
- Read optical densities (OD) at 405 nm

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Specific tests: *Indirect ELISA (IgG)*

- **Pre-coated ELISA plates: recomb. RVFV N-protein**
- **Blocking:** Add 100µl/well 10% skim milk in PBS for 1 h
- Wash three times with wash buffer;
- **Samples:** Add 50 µl samples diluted 1:100 in diluent buffer to wells in rows A-D: 1-12 and the corresponding wells in rows E-H: 1-12, incubate 37^{O}C for 1 h.
- Wash three times with wash buffer
- **Conjugate:** Add 50µl/well ready-made conjugate; incubate 37^{O}C for 1 h.
- Wash three times with wash buffer
- **Substrate:** Add 50 µl/well of ready-to-use TMB Substrate; incubated at room temperature in the dark for 20-30 minutes.
- **Stop reagent:** Add 50 µl of ready-to-use stop solution
- Read optical densities (OD) at 450 nm after 5 minutes.

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Specific tests: *Inhibition ELISA (IgG)*

- **Coating:** Coat plates with polyclonal sheep anti-RVF capture antibody diluted 1:400 in PBS, at +4^{O}C overnight.
- Wash three times with 0.1% Tween-20 in PBS;
- **Blocking:** with 200µl/well 10% skim milk in PBS at 37^{O}C for 1 h
- Wash three times with 0.1% Tween-20 in PBS;
- **Sample Pre-incubation:**, add 21µl of each undiluted test and control serum into diluting wells containing 189µl virus or control antigen pre diluted 1: 10 in 2% skim milk in PBS
- **Sample:** Add 100µl of each pre-incubated sample to rows A-D 1-12 and rows E-H 1-12 ; incubate 37^{O}C for 1 h.
- Wash three times with 0.1% Tween-20 in PBS;
- **Competitive antibody**: Add 100µl/well of mouse anti-RVF serum diluted 1:500 in diluent buffer; incubate 37^{O}C for 1 h
- Wash three times with 0.1% Tween-20 in PBS;
- **Conjugate:** Add 100µl /well of anti-mouse IgG HRPO-conjugate diluted 1:2000 in diluent buffer; incubate 37^{O}C for 1 h
- Wash six times with 0.1% Tween-20 in PBS;
- **Substrate:** .Add 100µl ABTS peroxidase per well ; incubated in the dark for 30 minutes at room temperature
- **Stop reagent:** SDS, Add 100µl
- Read optical densities (OD) at 405 nm

IAEA **Specific cut off's for each species !**

Specifics of the tests

Inhibition ELISA

Measure	Human	Cattle	Goat	Sheep	Buffalo	Camel
Cut-off	38.6 PI	41.9 PI	41.4 PI	38.4 PI	34.2 PI	36.1 IP
ensitivity (%)	99.47 VNT+[b] = 189	100 VNT+ = 59	99.56 VNT+ = 232	100 VNT+ = 65	100 VNT+ = 53	100 VNT+ = 75
pecificity (%)	99.66 VNT−[c] = 1178	99.52 VNT− = 635	99.65 VNT− = 574	99.29 VNT− = 428	99.51 VNT− = 205	100 VNT− = 81

IgM ELISA

sensitivity: 100%

Specificity	100	99.7	98.7

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RVF Sero-surveillance /Prevalence

- Domestic animals
  - Cattle, sheep goat — sandwich or indirect EIA
  - Camel (dog, rat?) — inhibition EIA
- Sentinel animals
  - Sheep (in most cases) — sandwich or indirect EIA
- Wildlife
  - Buffalos (others) — inhibition EIA
- Vaccination — none

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RVF outbreak

- Incidence
  - Cattle, sheep, goat — IgM capture EIA
  - Sentinel (~ monthly) — indirect, sandwich EIA
  - Camel, buffalo — ??
  - Wildlife — ??
- Diagnosis
  - Serology — AG-ELISA
  - Molecular — RT-PCR, LAMP
  - Virology — VNT, Isolation


Post outbreak / Trade

- **Today**:
  - 6 months after last clinical case
    - IgG test = **+** ➡ IgM testing (random) **-** ➡ fit f. export
    - **+** ➡ **PROBLEM**
- **Tomorrow**
  - 6 ? months after last clinical case
    - NSP test to differentiate vaccinated from infected

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The IAEA experience

- Coordinated Research Project on “Early diagnosis and control of RVF” started 2005
- Aim
  - Validate the commercialized serological tests
  - Evaluate the use of molecular tools in early diagnosis
- Countries
- Test results
- Recommendations

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RVF historic data

Table I. RVF IgG/IgM antibody prevalence in domestic ruminants in West Africa (1989-1992).

	Date	Species	Habitat	No.	RVF IgG %	CI 95	RVF IgM %
Cameroon	9-10/89	Sheep	Sa	63	20.63	9.4-28.7	0
		"	Su	280	9.26	5.9-12.7	0
		"	Gu	70	17.14	8.3-26.0	0
	9-10/89	Cattle	Su	156	10.25	5.5-15.0	0
		"	Gu	47	6.38	1.3-18.0	0
Togo	12/90-2/91	Sheep	Su	200	3.00	1.1-6.0	0
		"	Gu	127	0.79	0.0-4.0	0
	9-11/90	Cattle	Su	252	5.56	2.7-8.4	0
		"	Gu	143	16.08	10.1-22.1	0
Benin	12/91	Sheep	Su	236	0	0.0-2.0	0
		"	Gu	470	2.55	1.1-4.0	0
	12/91	Cattle	Su	130	6.15	2.7-12.0	0
			Gu	270	27.78	22.4-33.1	0
Ivory Coast	9/90	Sheep	Su	232	5.60	2.6-8.6	0.86
	10/90	"	Gu	513	9.16	6.7-11.7	0.97
	12/91-2/92	"	Gu	304	10.20	6.8-13.6	0.99
Burkina-Faso	11/90-1/91	Sheep	Sa	1,175	1.96	1.2-2.7	0
		"	Su	220	0.45	0.0-3.0	0
Senegal	9/91	Sheep	Sa	600	6.17	4.2-8.1	0
Total		Sheep	Sa	1,838	3.97	3.0-4.8	0
		"	Su	1,168	3.94	2.8-5.1	0.17
		"	Gu	1,484	6.94	5.6-8.2	0.53
	Total	"		4,490	4.73	3.7-4.9	0.22
		Cattle	Su	538	7.06	4.9-9.2	0
		"	Gu	460	21.96	18.2-25.7	0
	Total			998	13.93	11.8-16.1	0

Sa=Sahelian bioclimatic zone, Su=Sudanian, Gu=Guinean.
Ci=confidence interval.

Assays: Meegan, 1987

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Zeller et. al., Res. Virol. 1995, 146

RVF Results from 2006

		N. samples	IgG	IgM	Comment
Burkina Faso	Cattle		15%		in some regions only cattle +; Transhumans?
	sheep		7%		
	goat		4%		
Dem Rep. Congo	cattle	151	48%		difficult to interpret
Gambia	sheep	826	22%		
	goat	772	11%		
Guinea	sheep	471	3,3%	1,6%	
	goat	217	1,7%		
Kenya	sheep		24%		
	goat		20%		
Mauritania	sheep	348	15	0,6%	sentinel herds
Mali	sheep	816	4,4%		0 - 14% in 8 locations
Senegal	sheep	260	6%		sentinel
Uganda	sheep		20-50%		
Yemen	cattle		8,7%		including imported
	sheep		5%		
	goat		1,5%		

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Sentinel testing

Année	Nbre ser. testé	IgG+	% pos.	Nbre ser. testé	IgM+	% pos.
2002	0	0	-	538	11	2
2003	361	65	18	411	53	13
2004	0	0	-	520	7	1
2005	542	85	16	542	3	0,5
2006	583	56	10	583	4	0,6

Région	Site	Première visite			Deuxième visite			Troisième visite		
		Nb sérums	IgG+	IgM+	Nb sérums	IgG+	IgM+	Nb sérums	IgG+	IgM+
Assaba	Kankossa	28	2	0	15	0	1	0	0	0
Assaba	Kiffa	30	10	0	18	0	0	0	0	0
Brakna	Boghe	30	5	0	0	0	0	0	0	0
Chargui	Djiguenni	30	0	0	0	0	0	0	0	0
Chargui	Nema	21	0	0	30	0	0	0	0	0
Gharbie	Kobenni	29	1	0	27	0	0	0	0	0
Gharbie	Tintane	30	0	0	10	0	0	13	0	0
Gorgol	M'Bout	30	0	0	15	0	0	0	0	0
Guidimaka	Ghabou	30	1	0	27	1	0	0	0	0
Tagant	Tijikja	30	9	0	21	5	1	30	6	0
Trarza	K'Macene	30	1	0	15	0	1	0	0	0
Trarza	R'Kiz	28	14	0	16	1	1	0	0	0
Total :		**346**	**43**	**0**	**194**	**7**	**4**	**43**	**6**	**0**

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IgM

Table 2. *Prevalence of anti-RVFV IgM antibodies in sheep's, goats' and cattle sera from different farms in Kenya visited in 2005 to 2006*

Area	No. of serum samples Collected and tested			No. of serum samples found positive by IgM capture ELISA			Prevalence (%)		
	Sheep	Goats	Cattle	Sheep	Goats	Cattle	Sheep	Goats	Cattle
Molo	77	35	0	13	3	0	16.88	8.57	0
Rongai	34	86	0	17	17	0	50	19.77	0
Nakuru	53	39	0	4	1	0	7.55	2.56	0
Subukia	76	18	0	5	0	0	6.58	0	0
Naivasha	145	145	0	15	2	0	10.34	1.38	0
Kajiado	132	46	0	7	1	0	5.3	2.17	0
Namanga	36	41	0	1	0	0	2.78	0	0
Magadi	63	31	0	0	0	0	0	0	0
Shompole	14	14	0	0	1	0	0	7.14	0
Makueni	18	41	60	0	10	19	0	24.39	31.67
Kitui	11	37	0	2	8	0	18.18	21.62	0
Machakos	10	25	0	1	8	0	10	32	0
Kakamega	27	16	0	5	0	0	18.52	0	0
Kisumu	0	46	0	0	5	0	0	10.87	0
Lugari	32	37	0	3	5	0	9.38	13.51	0
Lamu	13	101	0	2	9	0	15.38	8.91	0
Malindi	14	40	0	2	9	0	14.29	22.5	0
Garissa	0	0	8	0	0	1	0	0	12.5


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QA data for 2006 IgG ELISA

Sample			Lab 1			Lab 2			Lab3			Lab 4			Lab 5			Lab6
no.	OVI	SNT-Bhk	PLATE 1	PLATE 2	PLATE 3	PLATE 1	PLATE 2	PLATE 3	PLATE 1	PLATE 2	PLATE 3	PLATE 1	PLATE 2	PLATE 3	PLATE 1	PLATE 2	PLATE 3	PLATE 1
1	POS	1:32	29	17	22	50	29	24	29	23	30	3	2	2	29	21	30	60
2	POS	1:12	16	10	9	24	21	15	10	13	10	13	14	9	10	14	10	97
3	POS	1:128	35	19	25	55	27	14	29	34	29	29	35	26	29	39	29	26
4	POS	1:12	18	9	7	22	12	19	13	32	12	13	18	10	13	14	12	43
5	POS	1:12	19	11	15	21	24	21	19	20	22	19	18	16	19	19	22	25
6	POS	1:64	39	21	26	42	28	18	31	28	28	36	39	23	31	44	28	3
7	POS	1:32	17	11	19	19	17	9	20	21	22	22	21	14	20	30	22	101
8	POS	1:512	66	57	49	94	50	58	56	64	57	83	69	53	56	86	57	50
9	POS	1:12	18	12	18	28	18	18	19	17	21	25	19	15	19	17	21	14
10	neg	neg	3	1	1	14	2	2	3	4	1	5	5	2	3	2	1	7
11	neg	neg	4	2	3	2	0	3	2	2	3	3	3	3	2	4	3	
12	neg	neg	4	3	2	5	6	6	4	3	2	4	6	5	4	4	5	
13	POS	1:128	25	20	22	24	24	25	26	20	22	26	31	23	26	31	26	!
14	POS	1:128	33	27	26	91	31	76	32	27	26	46	39	26	32	21	32	!
15	POS	1:24	12	5	8	31	14	11	6	5	8	10	11	13	6	10	9	!
16	susp	1:6	9	3	5	16	14	20	7	3	5	12	10	8	7	4	7	!
17	susp	neg	8	5	5	15	6	10	5	5	5	8	8	4	5	5	6	!
18	susp	neg	7	2	0	22	12	2	5	2	0	7	7	6	5	2	5	!
19	Neg	neg	9	5	1	12	4	15	4	5	1	7	4	5	4	6	3	!
20	neg	neg	7	4	0	13	4	5	4	4	0	7	5	4	4	5	6	!

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What are the problems observed

- Reference sera are not in the specified range => QC problem
- Too high variability of ref. sera results.
- Too labour intensive (double antigen)
- Missing reagents

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What do we need in future

- Simpler serology tests
  - Less steps
  - Expression antigen based, pre-coated
  - Better QC of kits
- DIVA test for trade
- Field test for early diagnosis

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What is in the pipeline

- N-Protein based indirect ELISA
  - IgG already under validation (human data publ.)
- N-Protein based IgM capture ELISA
  - IgM under evaluation
- Nss based ELISA for ruminant IgG
  - As future vaccines will have a deletion....
- IgM capture ELISA with rec.N-HPO
  - Less steps, much quicker
- Lateral flow test


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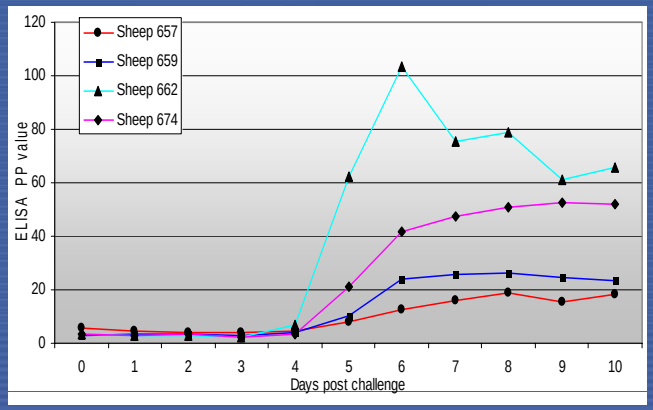
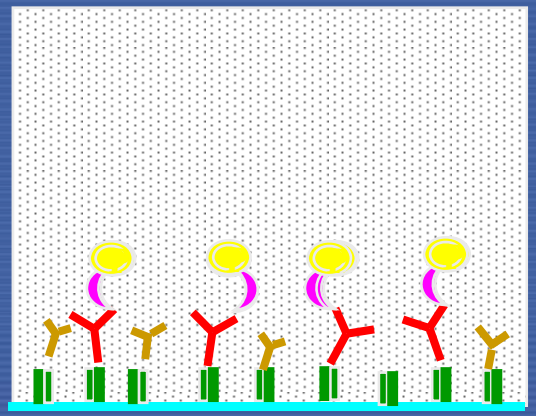
RVF N-Protein based IgM capture ELISA

- Coating with anti IgM (precoated)
- Samples undiluted
- N-HPO protein
- Substrate



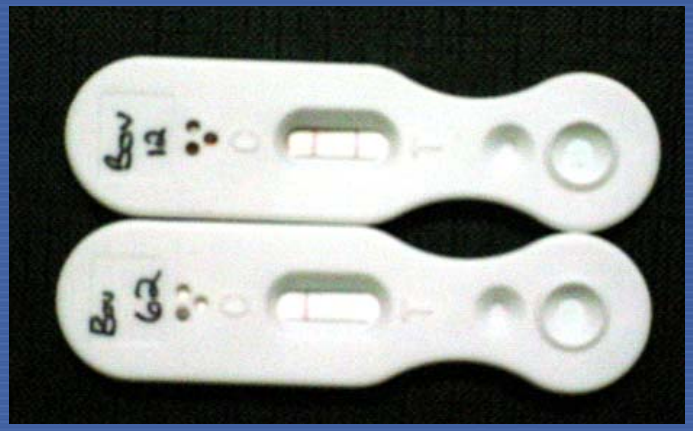


Design and results from OVI/ARC

RVF- Ab Lateral Flow test

Preliminary results from OVI/ARC

	RVF-ICT		
	Positive	Negative	
Infected	106	0	106
Non-infected	4	176	180
	110	176	



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And thanks to all these people!

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Trade

- Importing countries of meat or animals from RVF risk areas should not apply trade restrictions if disease surveillance is in place in countries/zones at **risk** with negative results.
- The OIE standards clearly separate
  - "**RVF infection free country or zone**" the country is out of the historical distribution area, a surveillance program is in place, and 4 years have elapsed since the last epidemic
  - "**RVF infected countries/zones without disease**". the country is considered free but disease has not occurred in humans and animals in the past 6 months and climatic changes predisposing to outbreaks of RVF have not occurred during this time.
  - "**RVF infected country/zone with disease**", meaning clinical disease in humans and animals has occurred within the past 6 months.
- Even in presence of disease or infection, the OIE Terrestrial Animal Health Code accepts trade of ruminants and meat from infected countries if certain specific conditions of quarantine or vaccination are met. This is based on timely and prompt notification of infection or disease to the OIE

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