EARLY DETECTION OF RIFT VALLEY FEVER
ANIMALS vs HUMANS vs VECTORS

R SWANEPOEL FOR:
PETRUS VAN VUREN, CHANTEL LE ROUX, JANUSZ PAWESKA
SPECIAL PATHOGENS UNIT
NATIONAL INSTITUTE FOR COMMUNICABLE DISEASES
SOUTH AFRICA

RIFT VALLEY FEVER: HISTORY/1

- 1910-12: DISEASE COMPATIBLE WITH RVF DESCRIBED IN LAMBS (EUROPEAN BREED) IN RIFT VALLEY, KENYA

- 1930: VIRUS FIRST ISOLATED IN OUTBREAK OF SHEEP DISEASE IN RIFT VALLEY, KENYA
  - MOSQUITO TRANSMISSION DEMONSTRATED
  - BENIGN HUMAN DISEASE WITH TRANSIENT LOSS OF VISUAL ACUITY NOTED

DAUBNEY

- SUBSEQUENT RECOGNITION OF PRESENCE OF VIRUS IN MANY SUB-SAHARAN COUNTRIES – (NB NOT CONFINED TO RIFT VALLEY)

- 1944: ISOLATION OF RVF VIRUS IN SEMLIKI FOREST UGANDA (NO LIVESTOCK OR HUMANS IN VICINITY) – HENCE RVF ASSUMED TO BE ENDEMIC IN FORESTS WITH SPREAD TO GRASSLANDS AFTER HEAVY RAINS

- 1950-1: LARGE OUTBREAK IN SOUTH AFRICA – ASSOCIATED WITH PANS & VLEIS (DAMBOS) - OCULAR LESIONS RECOGNIZED

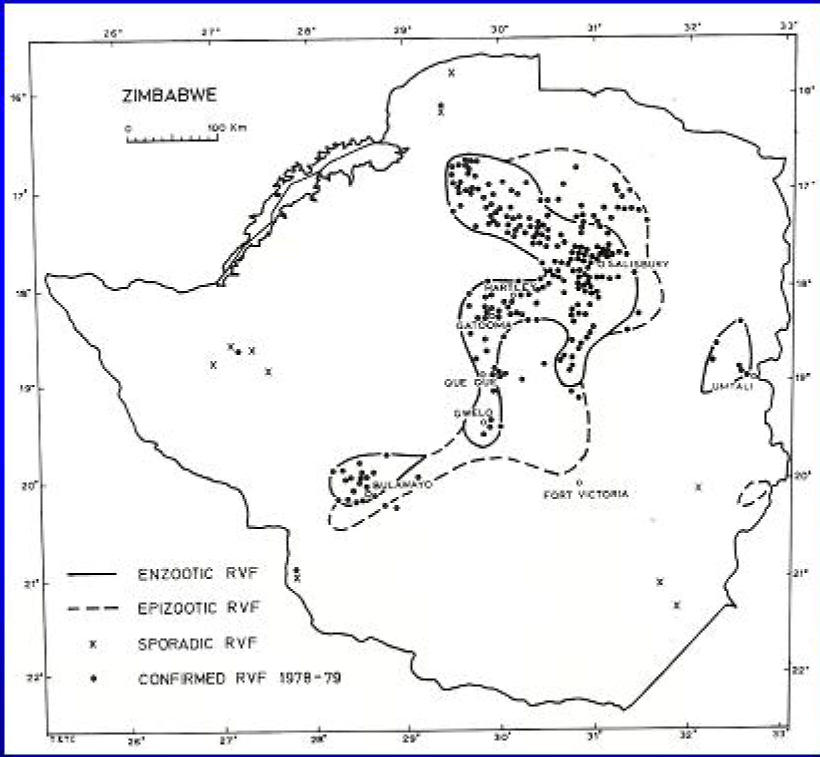
- 197-6: LARGE OUTBREAK IN SOUTH AFRICA – FATAL HUMAN DISEASE RECOGNIZED FOR FIRST TIME

RIFT VALLEY FEVER: HISTORY/2

- 1977-8: APPEARANCE OF RVF BEYOND SUB-SAHARAN AFRICA - IN EGYPT - >200,000 HUMAN INFECTIONS – 598 DEATHS
- 1979: RECOGNITION OF RVF IN MADAGASCAR
- 1987: LARGE OUTBREAKS IN MAURITANIA/SENEGAL – MANY HUMAN DEATHS
- 1997-8: LARGE OUTBREAK N-E KENYA/SOMALIA/TANZANIA - >300 HUMAN DEATHS
- 2000-1: APPEARANCE OF RVF BEYOND AFRICAN REGION IN SAUDI ARABIA & YEMEN – >200 DEATHS
- 2006-7: LARGE OUTBREAK N-E KENYA/SOMALIA/TANZANIA
- 2007: OUTBREAK IN SUDAN

RVF OBSERVATIONS IN ZIMBABWE 1955-1979

16,892 SERA, 4,002 VIROLOGICAL SPECIMENS FROM 2,354 LOCATIONS TESTED:



- RVF ENDEMIC IN SAVANAH/GRASSLANDS MAINLY ON CENTRAL WATERSHED PLATEAU

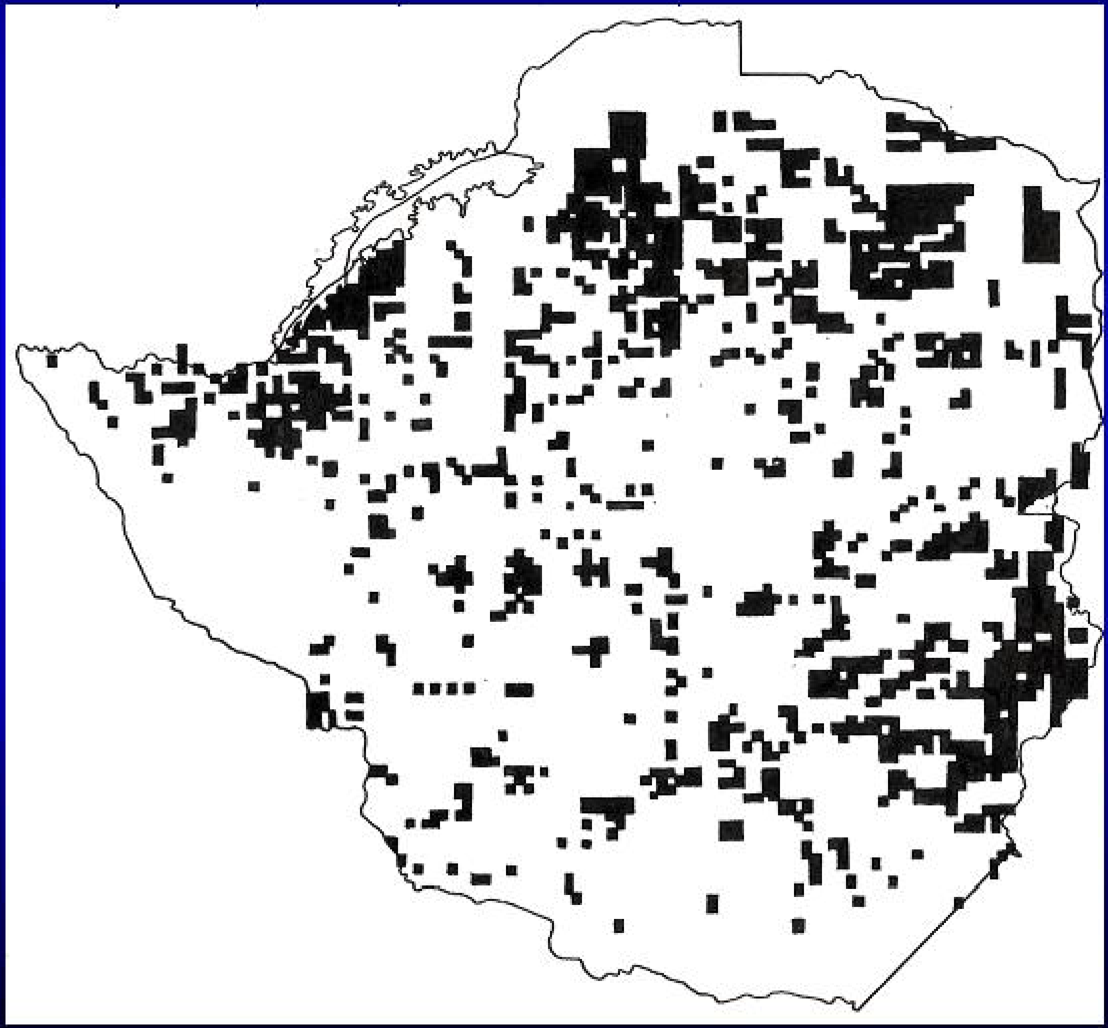
- LOW LEVEL OF VIRUS ACTIVITY TRIGGERED BY RAINS EVERY YEAR - ONLY DETECTED BY INTENSIVE MONITORING

- EPIDEMICS OCCUR IN SAME AREAS AS ENDEMIC VIRUS ACTIVITY - TRIGGERED BY EXCEPTIONAL RAINS

- ENDEMICITY PROBABLY ASSOCIATED WITH TRANSOVARIAL TRANSMISSION OF VIRUS IN FLOODWATER-BREEDING AEDES MOSQUITOES AS EARLIER DEMONSTRATED IN SOUTH AFRICA (1959) AND LATER IN KENYA (1985)

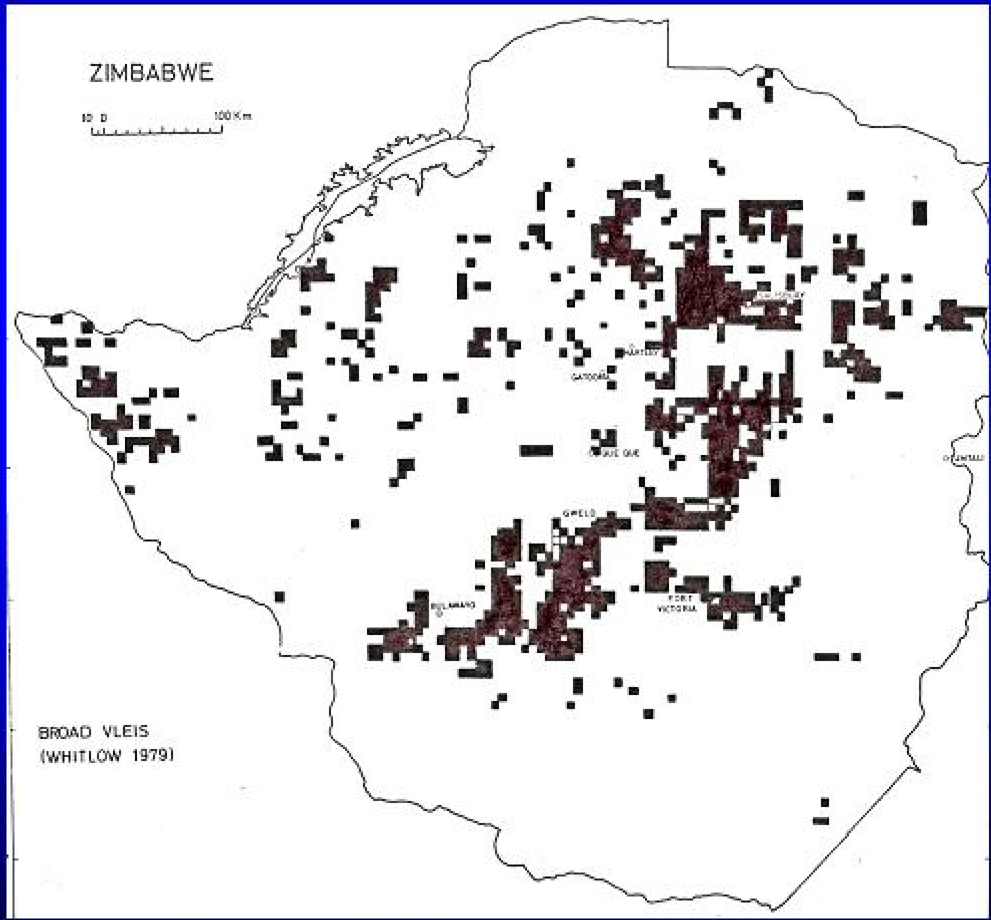
ZIMBABWE – CLOSED (CANOPY) FOREST – FROM AERIAL PHOTOGRAPHS

DISTRIBUTION OF FOREST DOES NOT CORRESPOND WITH RVF ENDEMICITY = MIRROR IMAGE



ZIMBABWE – DISTRIBUTION OF BROAD VLEIS (DAMBOS)

DISTRIBUTION OF BROAD VLEIS (DAMBOS) CORRESPONDS WITH AREAS OF RVF ENDEMICITY



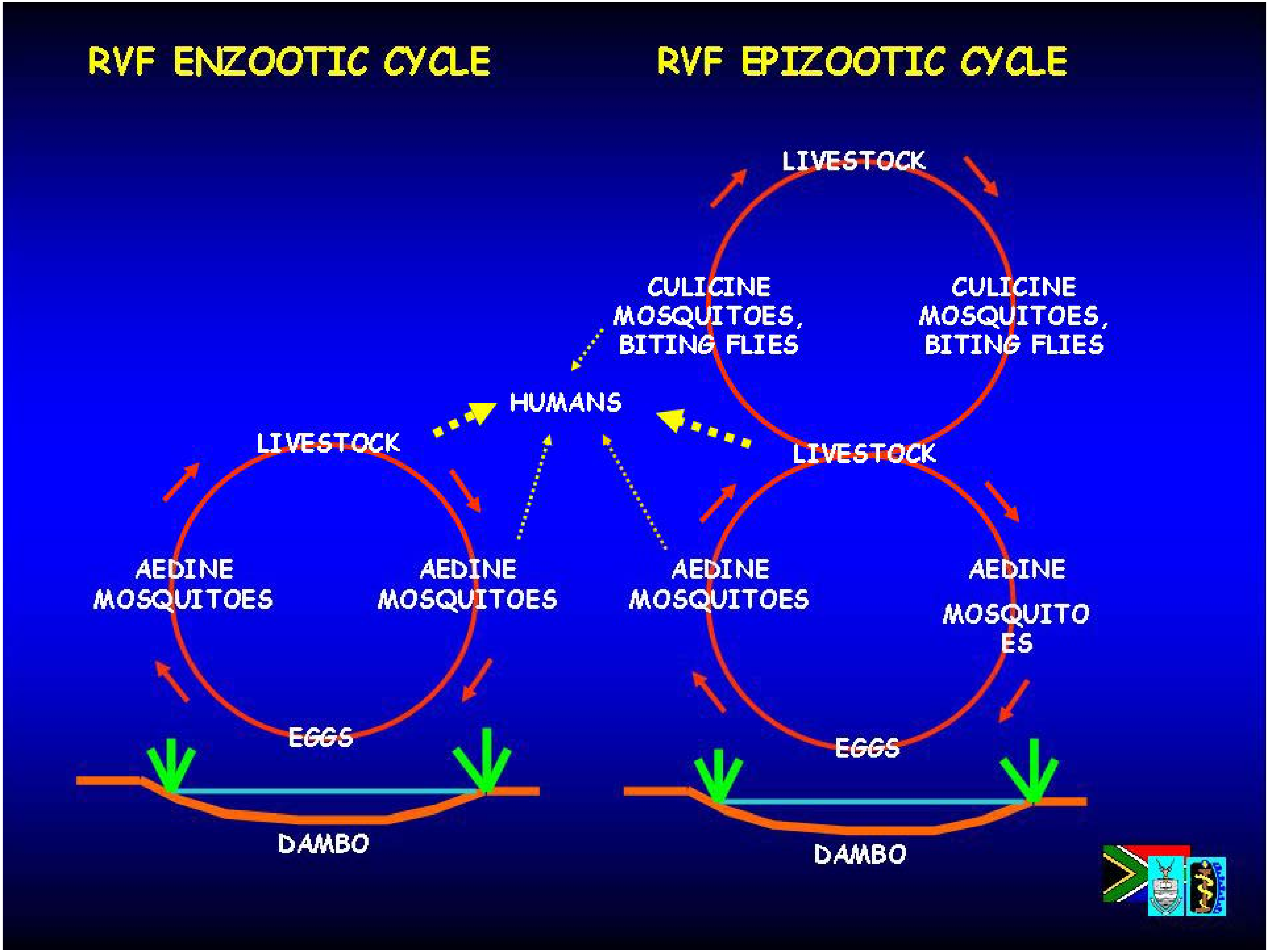
VECTORS OF RVF

ENZOOTIC (ENDEMIC) VECTORS = FLOODWATER-BREEDING AEDES MOSQUITOES (I.E. ONLY CERTAIN SPECIES OF AEDES)

- EGGS LAID IN MUD AT THE EDGE OF WATER IN FLOODED DAMBOS
- NB EGGS REQUIRE DRYING BEFORE THEY WILL HATCH WHEN THE DAMBOS BECOME FLOODED AGAIN
- EGGS CAN SURVIVE FOR YEARS IN DRY MUD
- TRANSOVARIAL TRANSMISSION OF VIRUS OCCURS IN A LOW PROPORTION OF INFECTED AEDES MOSQUITOES
- INFECTED EGGS = MECHANISM FOR PERPETUATION OF VIRUS
- INFECTED EGGS HATCH AND ADULT AEDES EMERGE AFTER FLOODING TO TRANSMIT INFECTION TO LIVESTOCK
- LIFE CYCLE RAPIDLY COMPLETED – 10-20 DAYS

EPIZOOTIC (EPIDEMIC) VECTORS = CULICINE MOSQUITOES, BITING FLIES

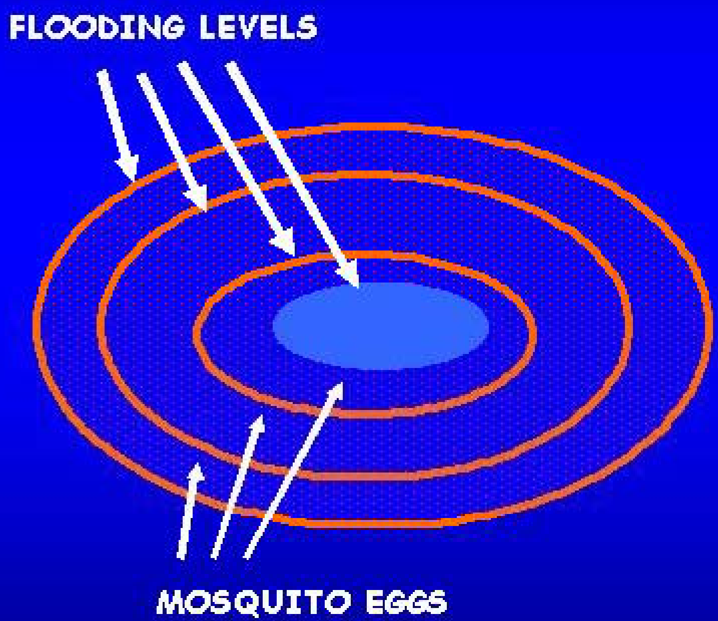
ACQUIRE VIRUS BY TAKING BLOODMEALS FROM INFECTED (VIRAEMIC) LIVESTOCK AND SUSTAIN THE OUTBREAK BY TRANSMITTING INFECTION

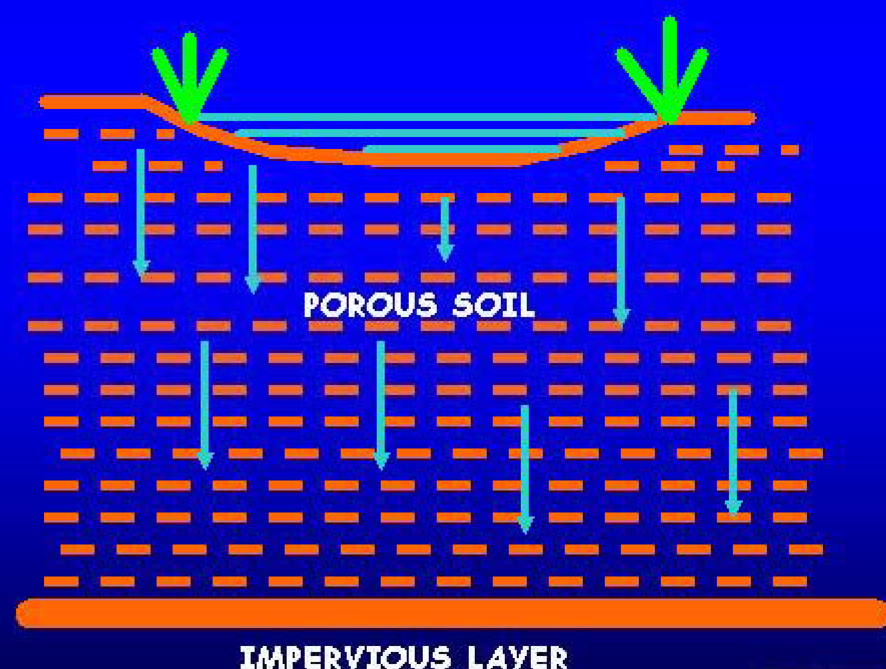


RVF ENZOOTIC CYCLE
RVF EPIZOOTIC CYCLE
LIVESTOCK
CULICINE MOSQUITOES, BITING FLIES
CULICINE MOSQUITOES, BITING FLIES
HUMANS
LIVESTOCK
LIVESTOCK
AEDINE MOSQUITOES
AEDINE MOSQUITOES
AEDINE MOSQUITOES
AEDINE MOSQUITO ES
EGGS
EGGS
DAMBO
DAMBO

DRAIANAGE/DRYING OR FLOODING OF DAMBOS:

EFFECTS ON FLOODWATER-BREEDING AEDES MOSQUITOES



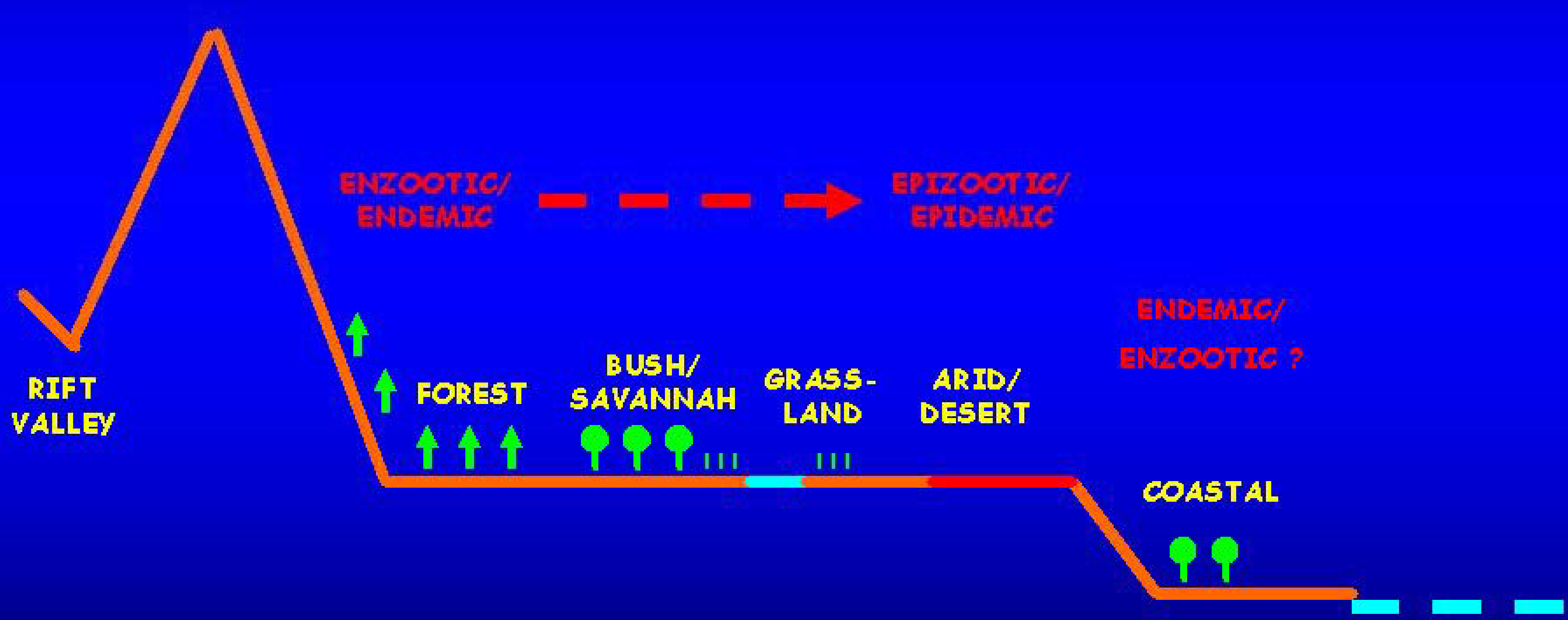


RVF VIRUS ISOLATIONS RECORDED IN KENYA 1961-98 ACCORDING TO ECOZONE (as summarised by Davies 1998)

	ECOZONE				
YEAR	II	III	IV	V	VI
1961	RVF	RVF	RVF	RVF	RVF
1962	RVF	RVF	RVF	RVF	
1963	RVF	RVF			
1967	RVF	RVF	RVF		
1968	RVF	RVF	RVF		
1971	RVF				
1977	RVF	RVF	RVF	RVF	
1978	RVF	RVF			
1981	RVF				
1983	RVF	RVF			
1989	RVF	RVF	RVF		
1990	RVF	RVF	RVF		
1993	RVF	RVF	RVF		
1994	RVF	RVF			
1997	RVF	RVF	RVF	RVF	RVF
1998	RVF	RVF	RVF	RVF	RVF

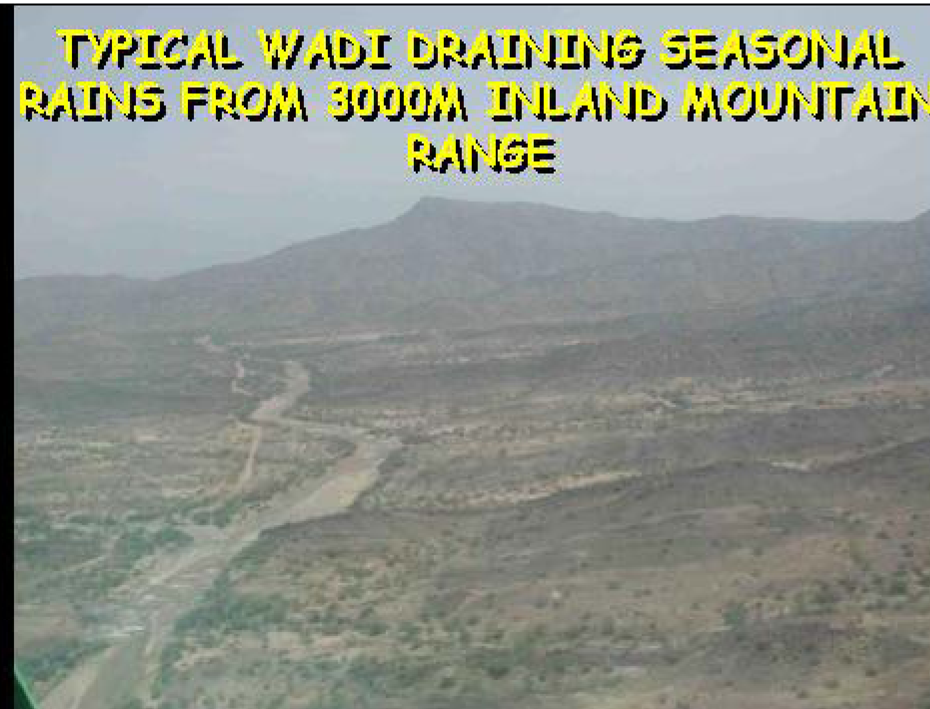
COCLUSION: RVF IS ENDEMIC IN KENYA
- ALSO MANY OTHER COUNTRIES IN AFRICA

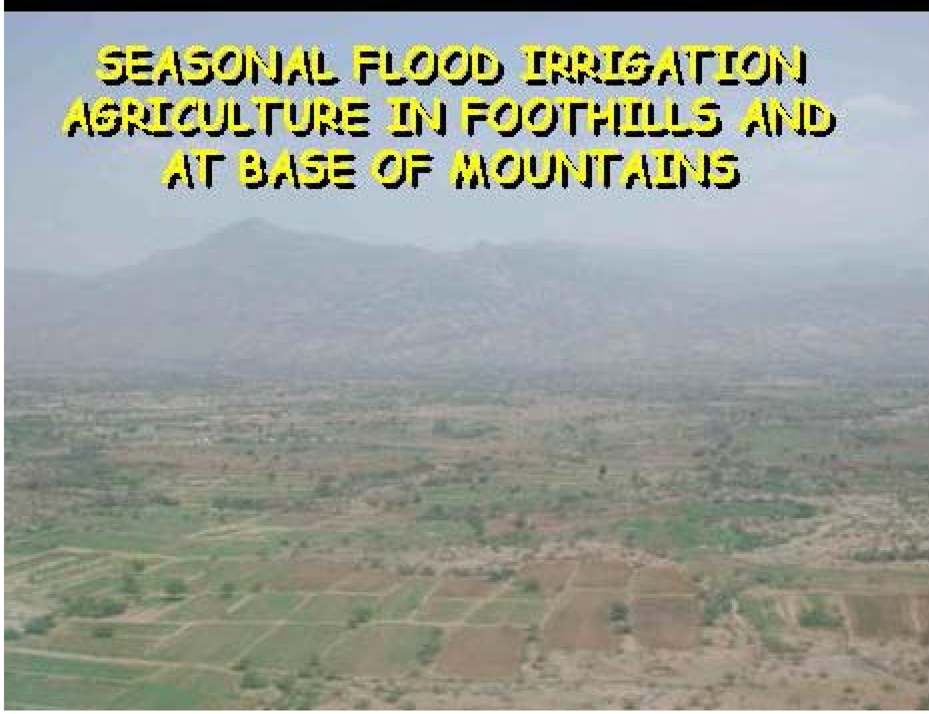
EFFECTS OF TOPOGRAPHY/ECOZONES ON RVF EPIDEMIOLOGY

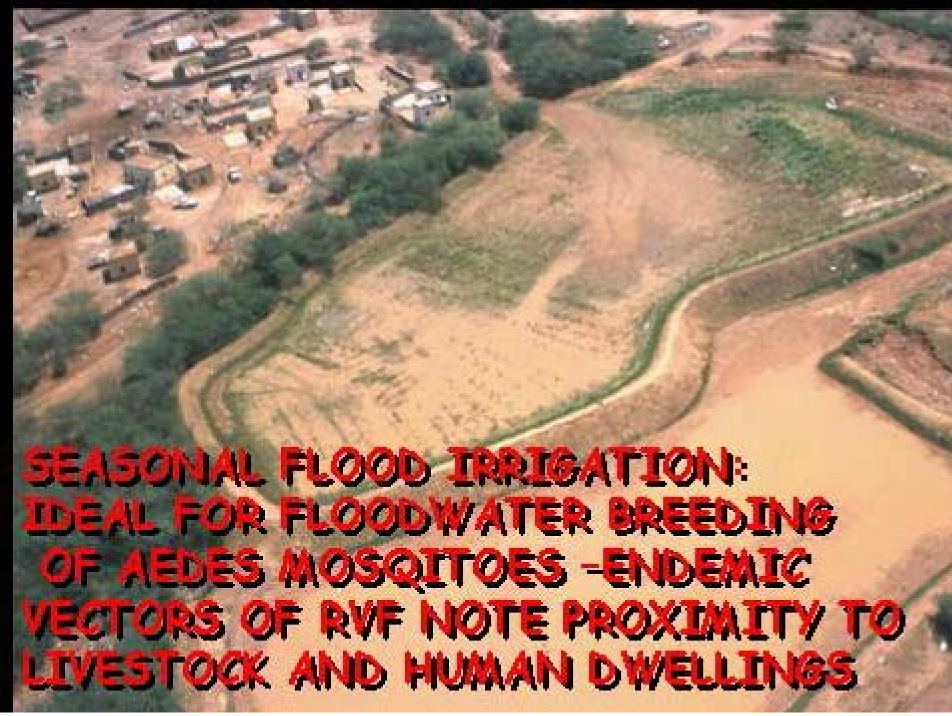


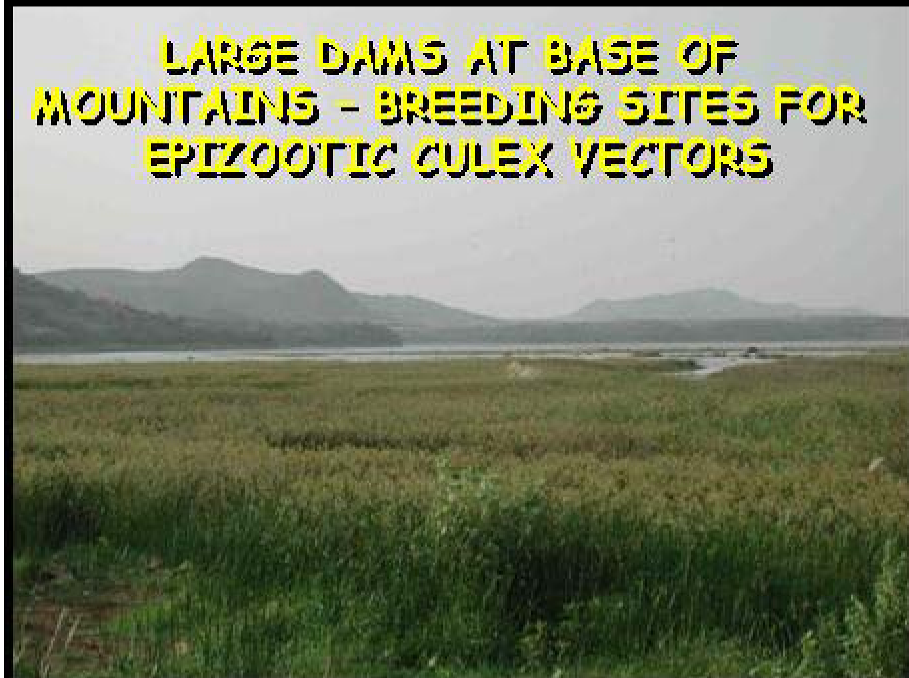
1997-98 RVF OUTBREAK
NE KENYA & SOMALIA


COASTAL TIHAMA DESERT NOT CONDUCIVE FOR RVF OUTBREAKS EXCEPT FOR WADIS DRAINING MOUNTAIN PRECIPITATION TO RED SEA

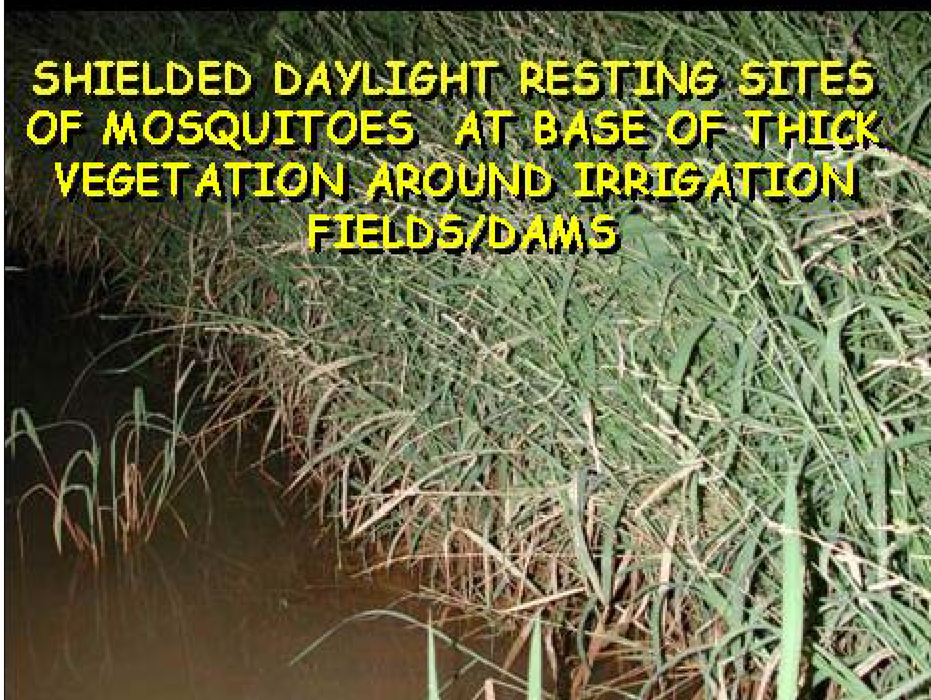

TYPICAL WADI DRAINING SEASONAL RAINS FROM 3000M INLAND MOUNTAIN RANGE


SEASONAL FLOOD IRRIGATION AGRICULTURE IN FOOTHILLS AND AT BASE OF MOUNTAINS

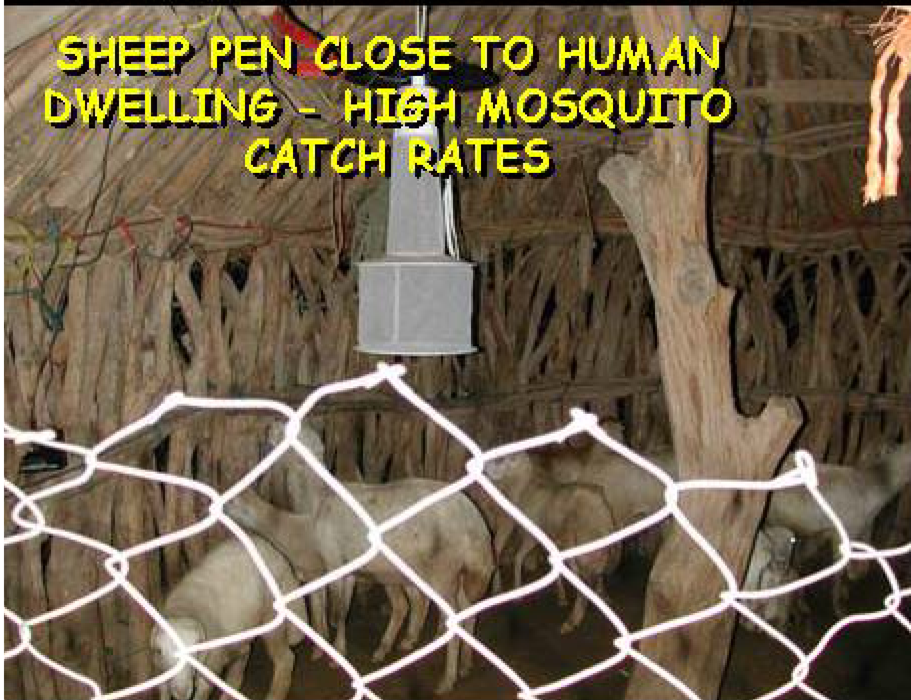

SEASONAL FLOOD IRRIGATION:
IDEAL FOR FLOODWATER BREEDING OF AEDES MOSQITOES –ENDEMIC VECTORS OF RVF NOTE PROXIMITY TO LIVESTOCK AND HUMAN DWELLINGS


LARGE DAMS AT BASE OF
MOUNTAINS - BREEDING SITES FOR
EPIZOOTIC CULEX VECTORS


INTENSIVE INSECTICIDE SPRAYING
BY AIRCRAFT, VEHICLES AND
HANDPUMPS


SHIELDED DAYLIGHT RESTING SITES
OF MOSQUITOES AT BASE OF THICK
VEGETATION AROUND IRRIGATION
FIELDS/DAMS

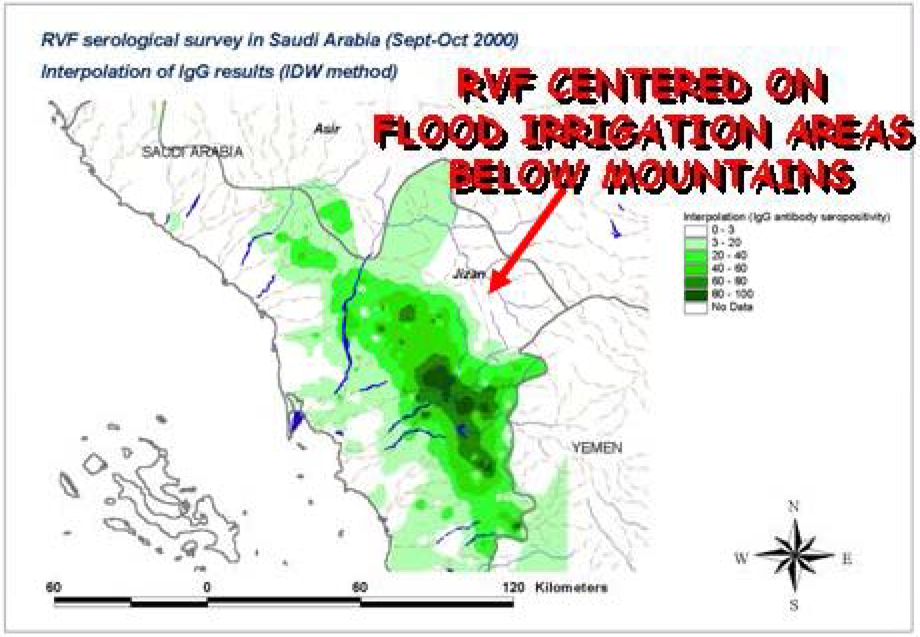

CATCH RATES OF 30,000 MOSQUITOES
PER TRAP NIGHT DESPITE AERIAL
SPRAYING

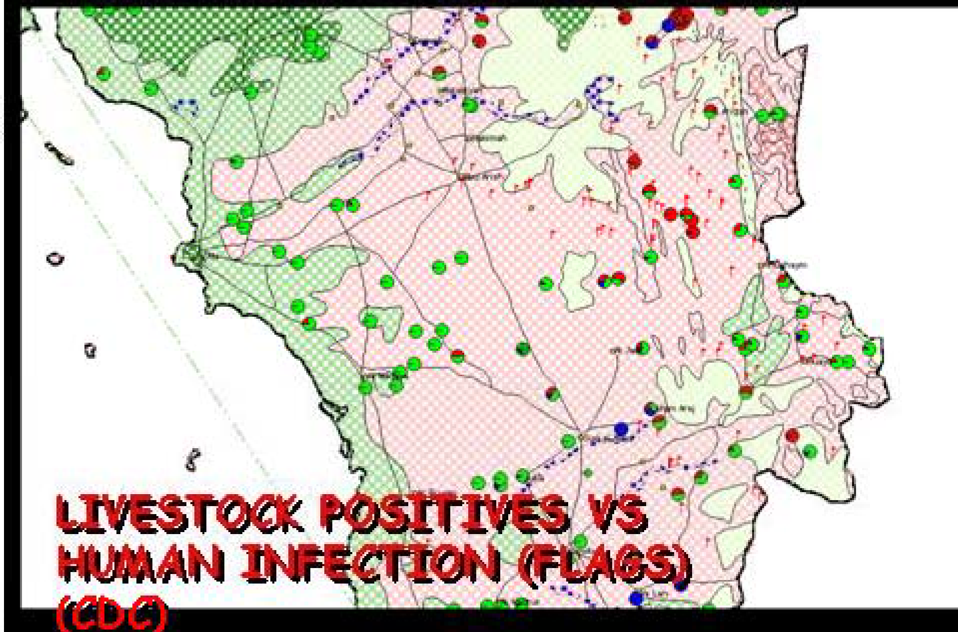

SHEEP PEN CLOSE TO HUMAN DWELLING – HIGH MOSQUITO CATCH RATES

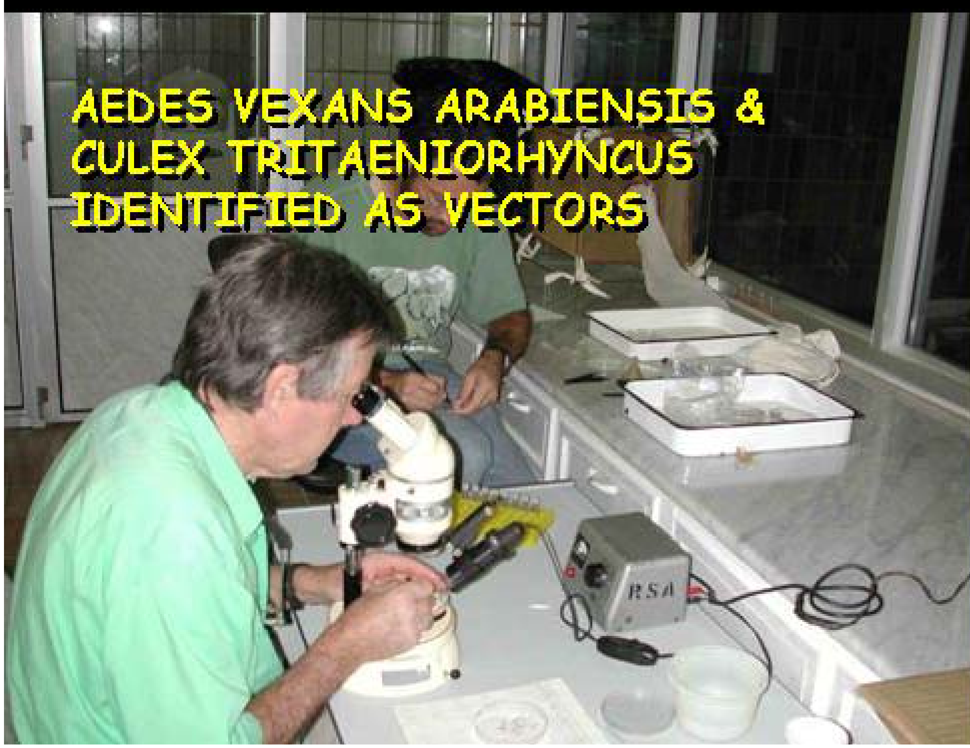

HEAT: PEOPLE SLEEP OUTDOORS – EXPOSED TO MOSQUITOES
HIGHER INFECTION RATES IN THE POOR WHO CANNOT AFFORD NETS AND FANS

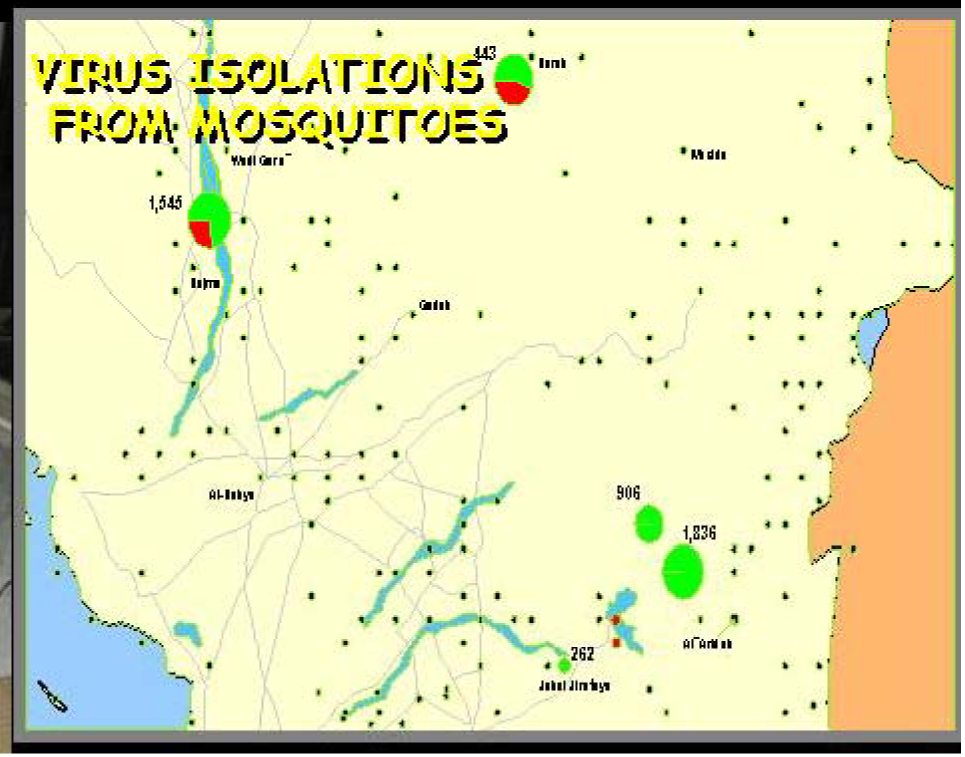

>200 DEATHS (WHO)


EMERGENCY LAB – 7000 SERA TESTED IN 2 WEEKS (ELISA IgG & IgM


RVF serological survey in Saudi Arabia (Sept-Oct 2000)
Interpolation of IgG results (IDW method)
RVF CENTERED ON
FLOOD IRRIGATION AREAS
BELOW MOUNTAINS
SAUDI ARABIA
Asir
Jizan
YEMEN
Interpolation (IgG antibody seropositivity)
0 - 3
3 - 20
20 - 40
40 - 60
60 - 80
80 - 100
No Data
60
0
60
120 Kilometers
N
W
E
S


LIVESTOCK POSITIVES VS
HUMAN INFECTION (FLAGS)
(CDC)


AEDES VEXANS ARABIENSIS &
CULEX TRITAENIORHYNCUS
IDENTIFIED AS VECTORS
RSA

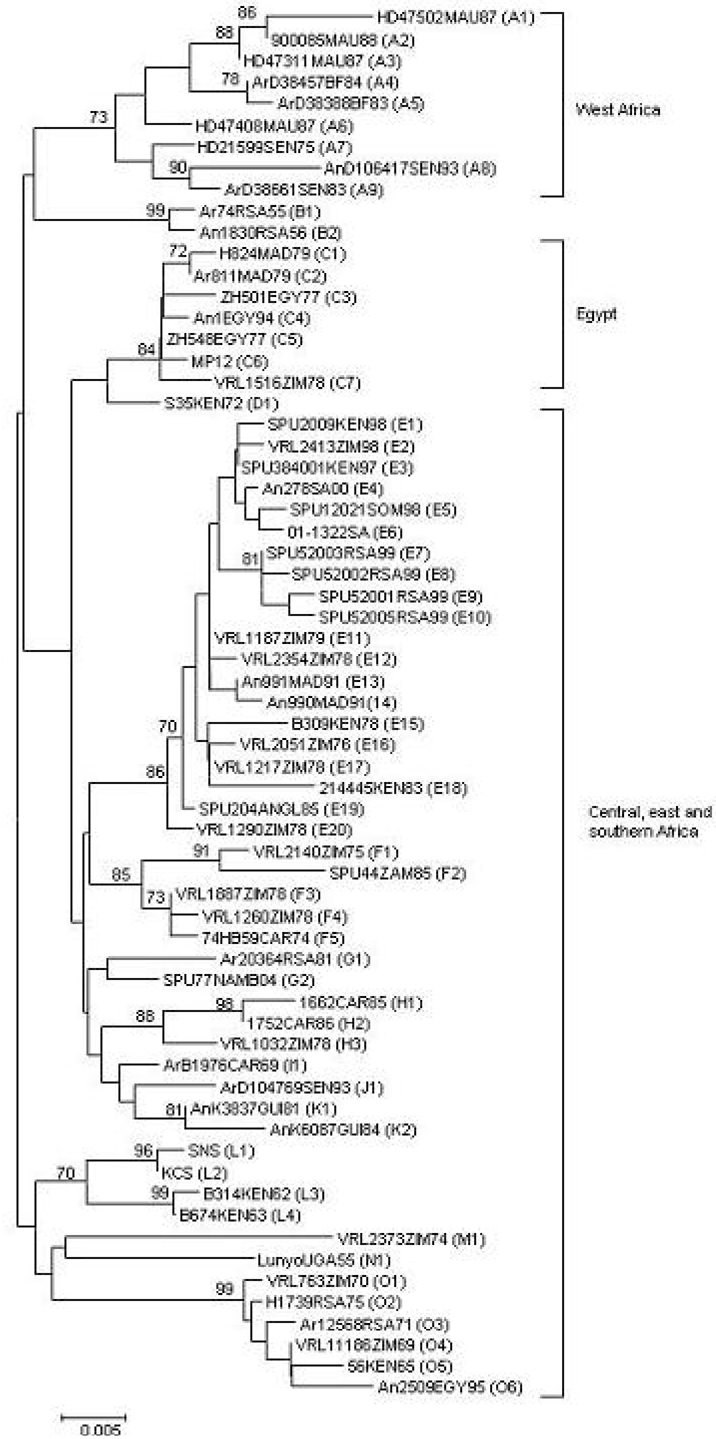

VIRUS ISOLATIONS
FROM MOSQUITOES
443
1,545
906
1,836
262

RVF VIRUS PHYLOGENY

- VIRUS REMARKABLY STABLE GENETICALLY AND ANTIGENICALLY

- FOLLOWING HEAVY RAINS OUTBREAKS ASSOCIATED EITHER WITH A SINGLE GENETIC VARIANT OF VIRUS (= EPIDEMIC SPREAD)
OR
WITH SIMULTANEOUS EMERGENCE OF MULTIPLE VARIANTS FROM ENDEMIC FOCI

(unpublished information NICD)



PHASES OF RVF DETECTION:

PREDICTION

REMOTE SENSING – RAINFALL ESTIMATES
- NDVI
- ENSO PHENOMENA

SURVEILLANCE

VETERINARY & MEDICAL ALERTNESS
SENTINEL HERDS/FLOCKS
VECTOR SURVEILLANCE

RECOGNITION

VETERINARY DISEASE
HUMAN DISEASE
(VECTORS)

CONFIRMATION

LABORATORY METHODS

RECOGNITION OF RVF OUTBREAKS

- SUDDEN OUTBREAK OF DISEASE INVOLVING DEATHS OF YOUNG RUMINANTS (ESPECIALLY LAMBS & CALVES) AND ABORTION IN PREGNANT ADULTS FOLLOWING THE OCCURRENCE OF HEAVY RAINS
- ACCOMPANIED BY REPORTS OF FEBRILE DISEASE IN HUMANS – OFTEN WITH SOME DEATHS
- OUTBREAKS OFTEN OCCUR IN AREAS WITH POOR ROADS AND SERVICES – ABORTIONS IN LIVESTOCK AT 5-15% LEVEL NOT REPORTED
- IN CONTRAST THE OCCURRENCE OF HUMAN DISEASE WITH FATALITIES IS USUALLY REPORTED BY MEDICAL SERVICES/NGO's

CLINICAL SIGNS IN LIVESTOCK

- YOUNG ANIMALS:
- SUDDEN ONSET OF HIGH FEVER
- ACUTE PROSTRATION, COLLAPSE & DEATH

- ADULTS:
- ABORTIONS THE MOST IMPORTANT SIGN
- DYSTOCIA, SOME TERATOLOGY, HYDROPS AMNII
- ANOREXIA, DYSGALACTIA, NASAL AND LACHRYMAL DISCHARGES
- SALIVATION, 'VOMITING', LYMPHADENITIS
- COLIC, JAUNDICE, HAEMORRHAGIC ENTERITIS

MORBIDITY/MORTALITY IN LIVESTOCK

- SHEEP MOST SUSCEPTIBLE → CATTLE → GOATS→ CAMELS LEAST SUSCEPTIBLE (ABORTION ONLY)
- CLINICAL DISEASE ESPECIALLY IN EXOTIC BREEDS
- INDIGENOUS ANIMALS GENERALLY LESS SUSCEPTIBLE – EXCEPT IN ARID ZONES
- 20-90% MORBIDITY
- 40-60% MORTALITY IN YOUNG, 2-5% IN ADULTS
- PREGNANT ANIMALS ABORT

RVF HUMAN DISEASE

INFECTED BY CONTACT WITH DISEASED ANIMAL TISSUES
OR
MOSQUITO BITE – LESS COMMON IN SUB-SHAHARAN AFRICA WHERE VECTORS ARE SYLVATIC (DO NOT ENTER DWELLINGS)

INCUBATION PERIOD <1 WEEK

=80% INFECTIONS SUBCLINICAL OR MILD

<0.5% FATAL HEMORRHAGIC FEVER/ENCEPHALITIS

APPROXIMATELY 5% OCULAR SEQUELAE

LABORATORY CONFIRMATION OF CURRENT RVF INFECTION

- ANATOMICAL PATHOLOGY
- ANTIGEN DETECTION (AGID, ELISA, IF)
- RT-PCR DETECTION OF VIRAL RNA
- VIRUS ISOLATION (MOUSE INOCULATION, TC)
- ANTIBODY TESTS (HAI, NEUT, ELISA IgM, IgG)
- HISTOPATHOLOGY, IMMUNOHISTOCHEMISTRY

VIROLOGICAL DIAGNOSIS OF RVF

ANTIGEN DETECTION

Recombinant NP sandwich ELISA

NUCLEIC ACID DETECTION

RT-PCR

Taqman Real-Time PCR

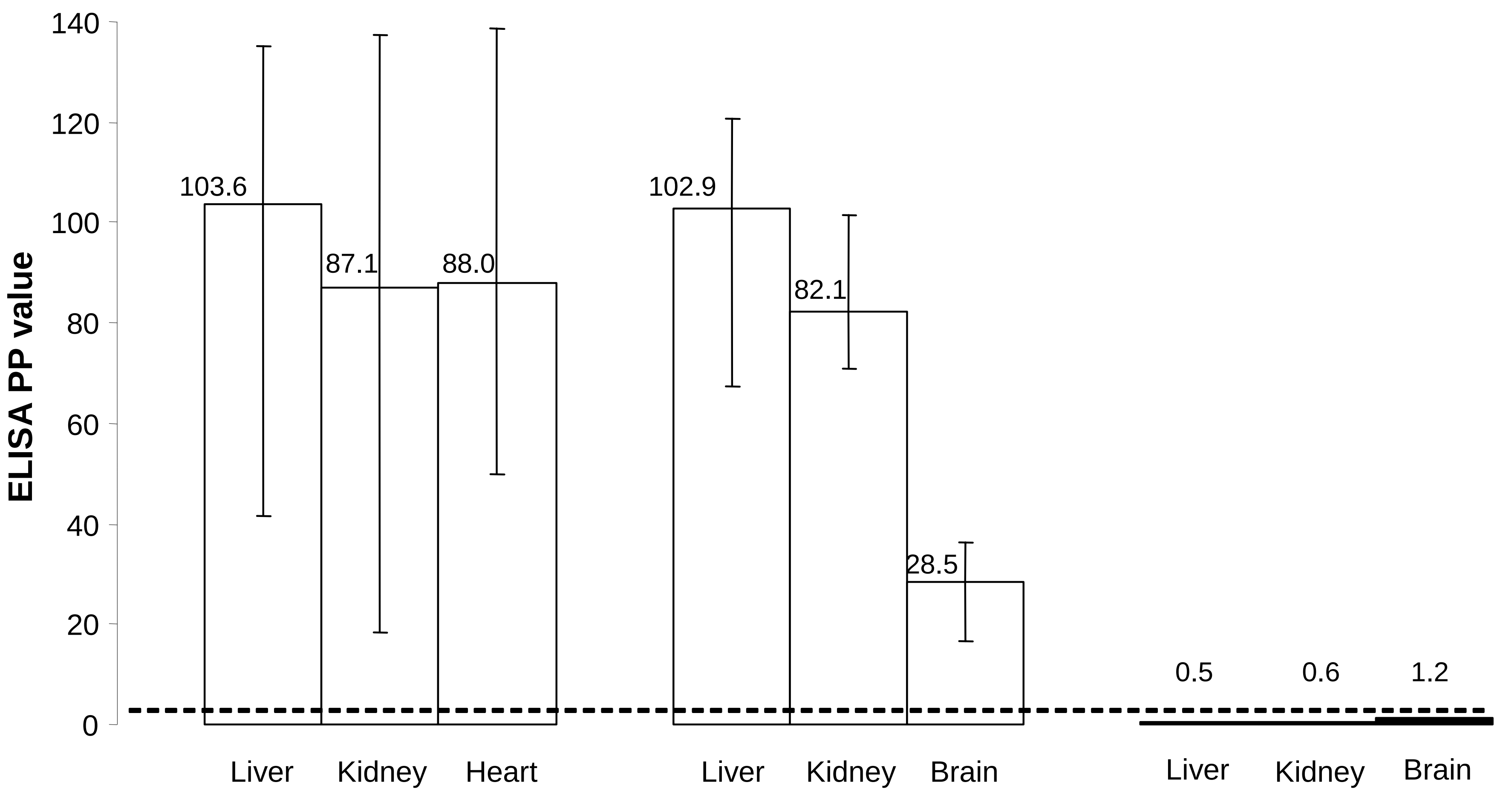
LAMP RT-PCR

VIRUS ISOLATION

Mice

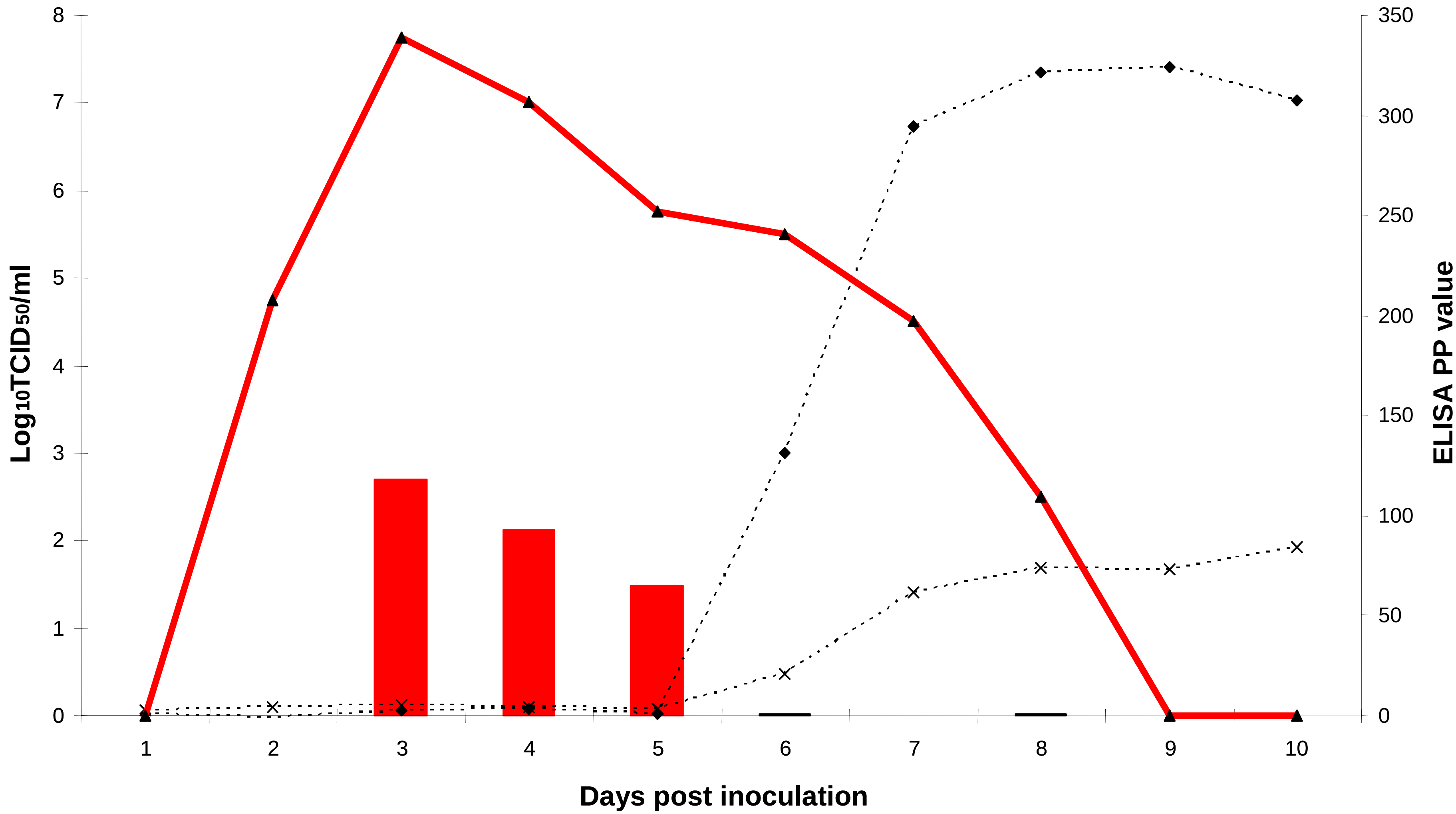
Cell cultures

ELISA DETECTION OF RVF ANTIGEN (NUCLEOCAPSID PROTEIN)



RVF-infected buffalo fetus | Infected mouse | Non-infected mouse

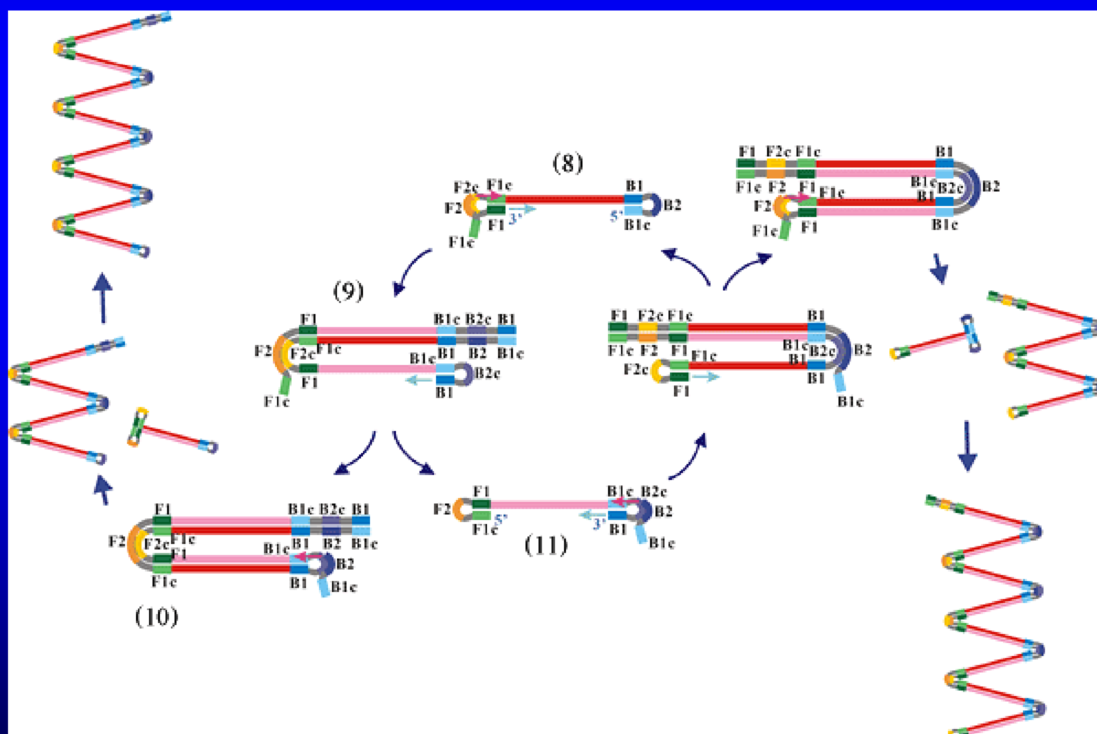
ELISA DETECTION OF ANTIGENEMIA



LOOP-MEDIATED ISOTHERMAL AMPLIFICATION (LAMP)

AMPLIFIES TARGET NUCLEIC ACID UNDER ISOTHERMAL CONDITIONS (60 - 65°C) USING SIMPLE EQUIPMENT: HEATING BLOCK OR WATER BATH (NOT THERMPCYCLER)

BASED ON AUTOCYCLING STRAND DISPLACEMENT DNA SYNTHESIS BY Bst DNA POLYMERASES AND 4-6 PRIMERS

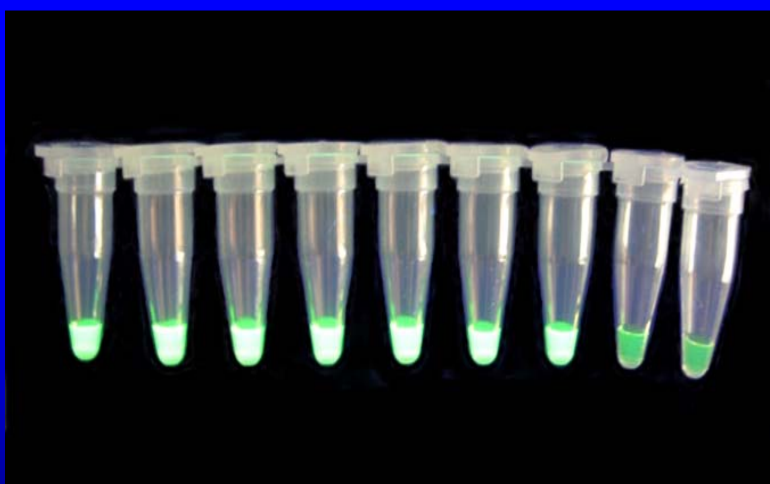


Courtesy of Prof Morita, University of Nagasaki, Japan

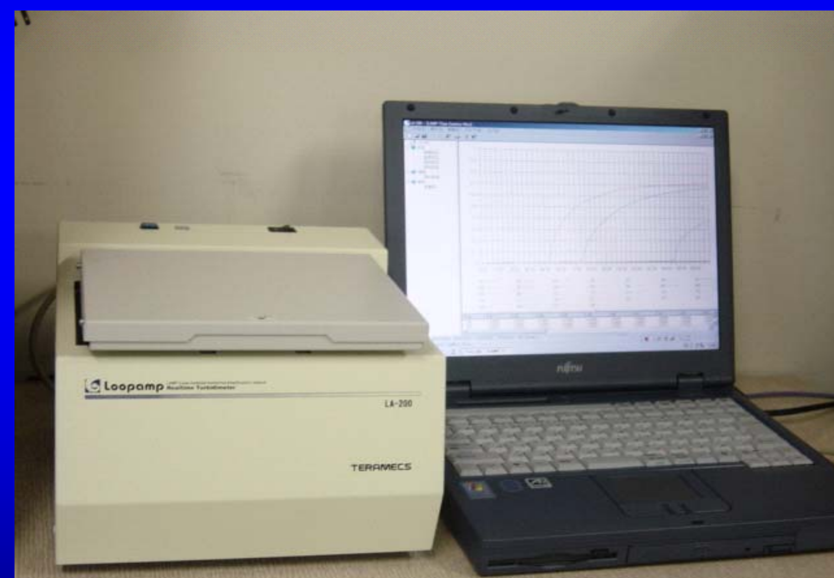
LOOP-MEDIATED ISOTHERMAL AMPLIFICATION (LAMP)

Products visible by naked eye, fluorescence, agarose gel electrophoresis, or turbidity

1 2 3 4 5 6 7 8 9



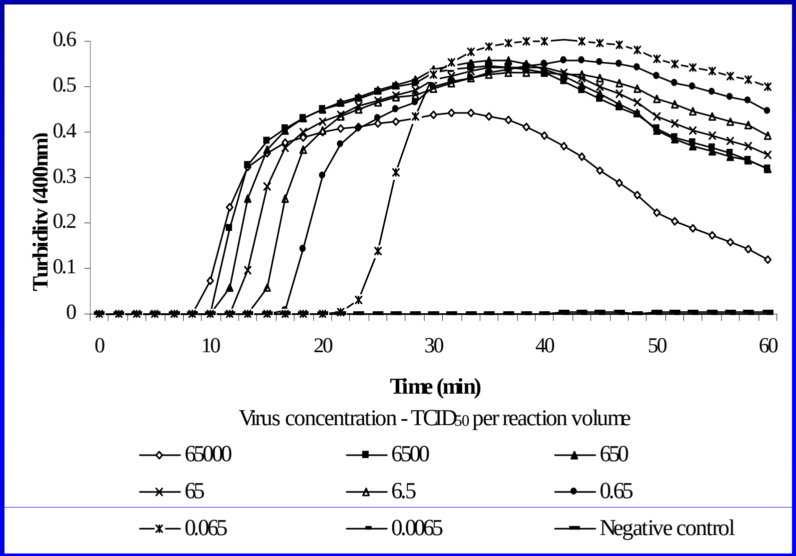
LAMP turbidimeter connected to laptop for real-time monitoring



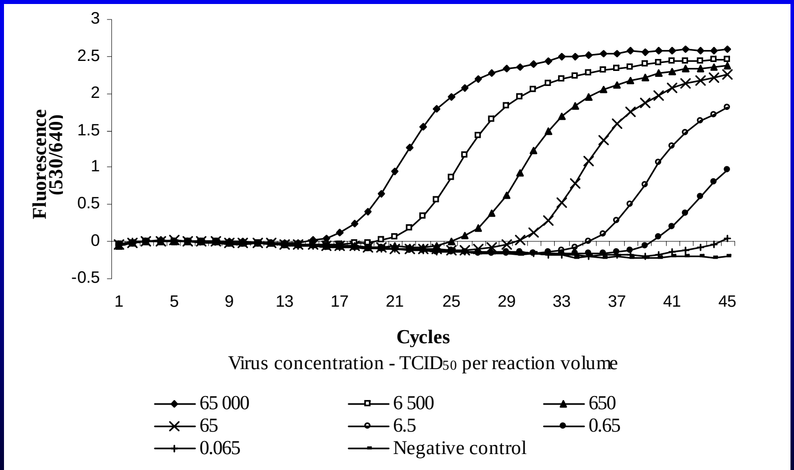
Courtesy of Prof Morita, University of Nagasaki, Japan

RT-LAMP ASSAY & Taqman RTD-PCR EQUALLY SENSITIVE: DETECTION LIMIT 0.065 TCID50/REACTION VOLUME

RT-LAMP



Taqman RT-PCR



COMPARISON OF RT-LAMP, Taqman-PCR & VIRUS ISOLATION FOR DETECTION OF RVFV IN CLINICAL SPECIMENS

Specimen	Source	Number tested	Results RT-LAMP + RTD-PCR + Isolation +	Results RT-LAMP - RTD-PCR - Isolation -	Results RT-LAMP + RTD-PCR + Isolation -	Results RT-LAMP - RTD-PCR - Isolation +
Serum	Sheep	20	10	10	0	0
Plasma	Sheep	6	6	0	0	0
Serum	Human	65	31	32	1	1
Liver	Buffalo	3	3	0	0	0
Kidney	Buffalo	3	3	0	0	0
Total		97	53	42	1	1

ELISA ANTIBODY TESTS

1. INACTIVATED MOUSE LIVER ANTIGEN, ANTI-SPP CONJUGATES:

HUMAN

Sandwich ELISA: anti-RVFV IgG in humans
Capture ELISA: anti-RVFV IgM in humans

LIVESTOCK

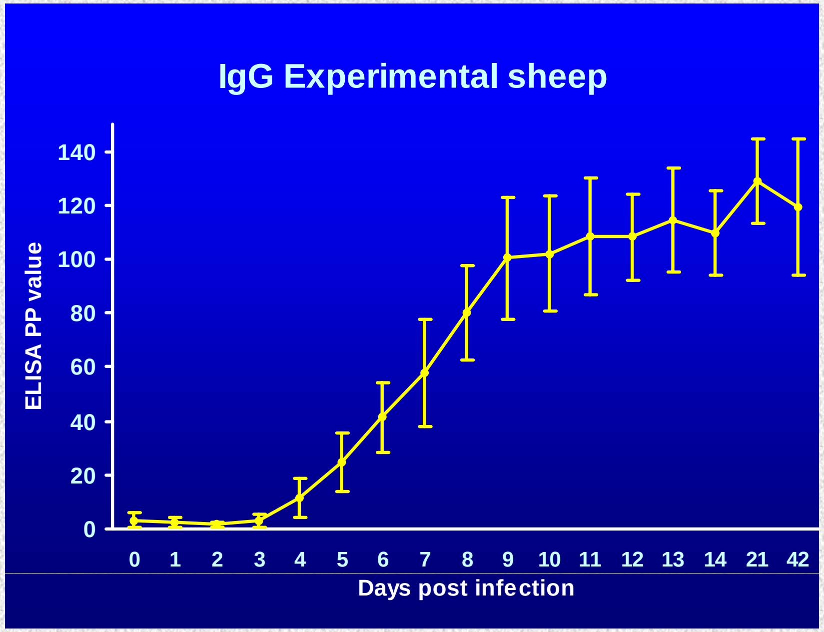
Sandwich ELISA: anti-RVFV IgG in cattle
Sandwich ELISA: anti-RVFV IgG in sheep and goats
Capture ELISA: anti-RVFV IgM in sheep, goats and cattle

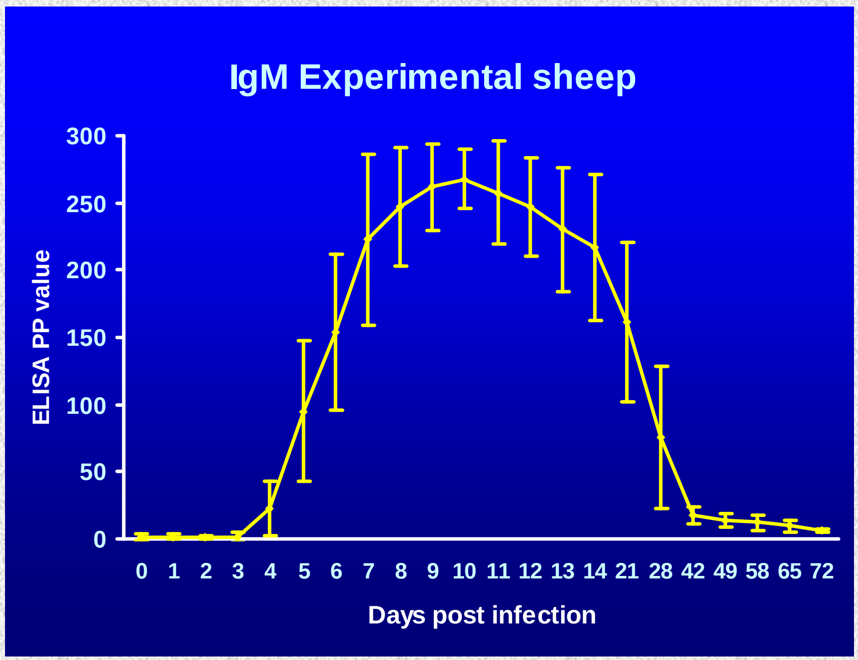
2. RECOMBINANT ANTIGEN, ANTI-PROTEIN G CONJUGATE:

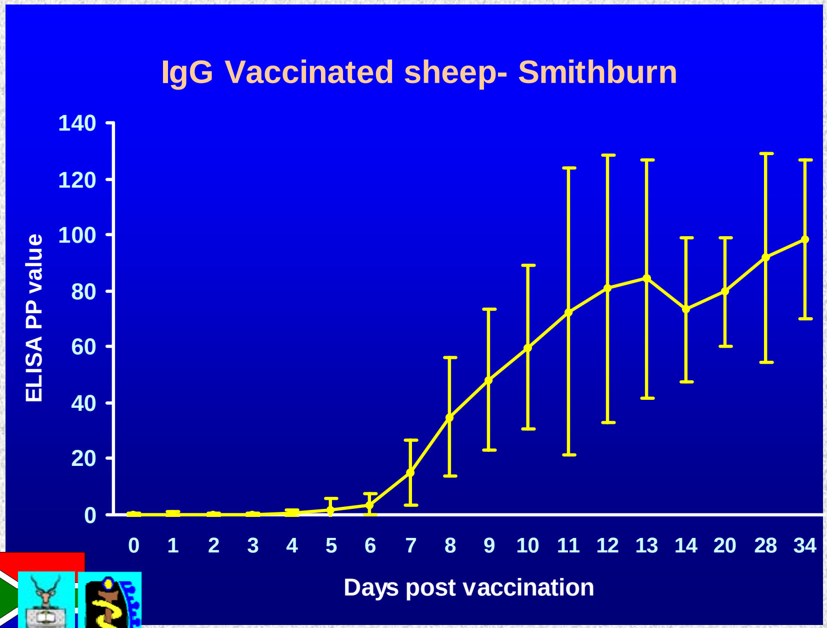
Recomb NP indirect ELISA: anti-RVFV IgG in sheep, goats and cattle
Recomb NP indirect ELISA: anti-RVFV IgG in wild ruminants
Recomb NP indirect ELISA: anti-RVFV IgG antibody in humans

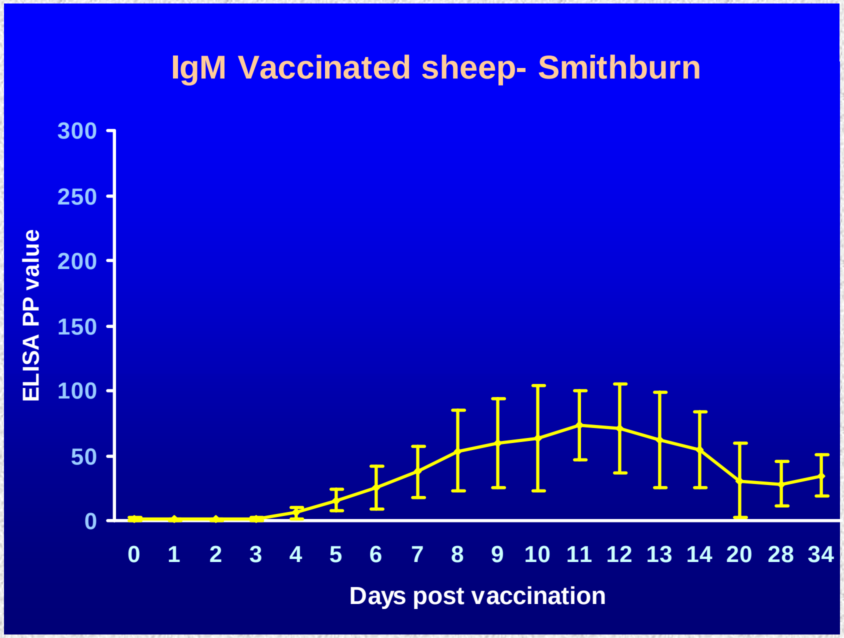
3. CELL-LYSATE ANTIGEN, DETECTION: RABBIT ANTI-RVF & CONJUGATE:

Inhibition ELISA: anti-RVFV in humans, domestic & wild animals


IgG Experimental sheep
ELISA PP value
0 20 40 60 80 100 120 140
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 21 42
Days post infection

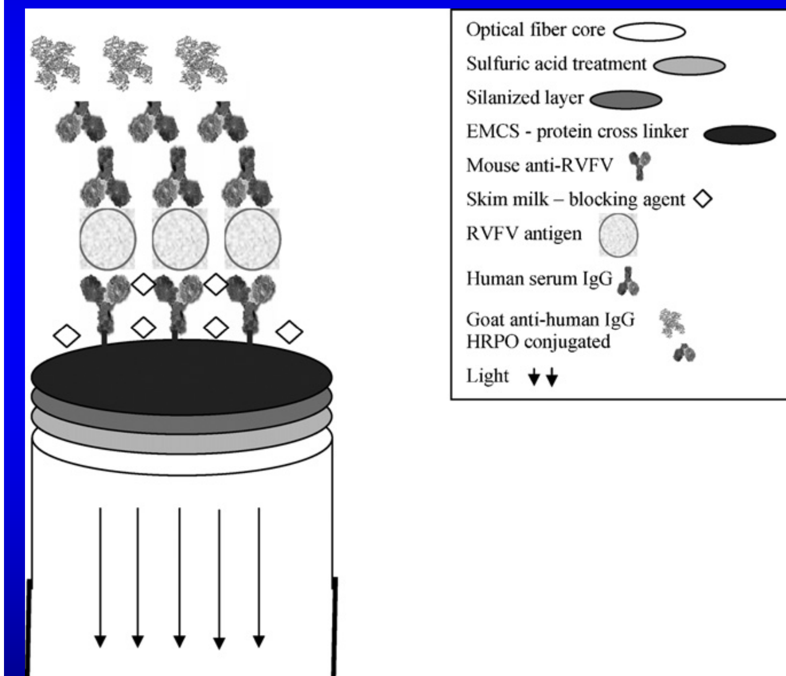

IgM Experimental sheep
ELISA PP value
0 50 100 150 200 250 300
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 21 28 42 49 58 65 72
Days post infection

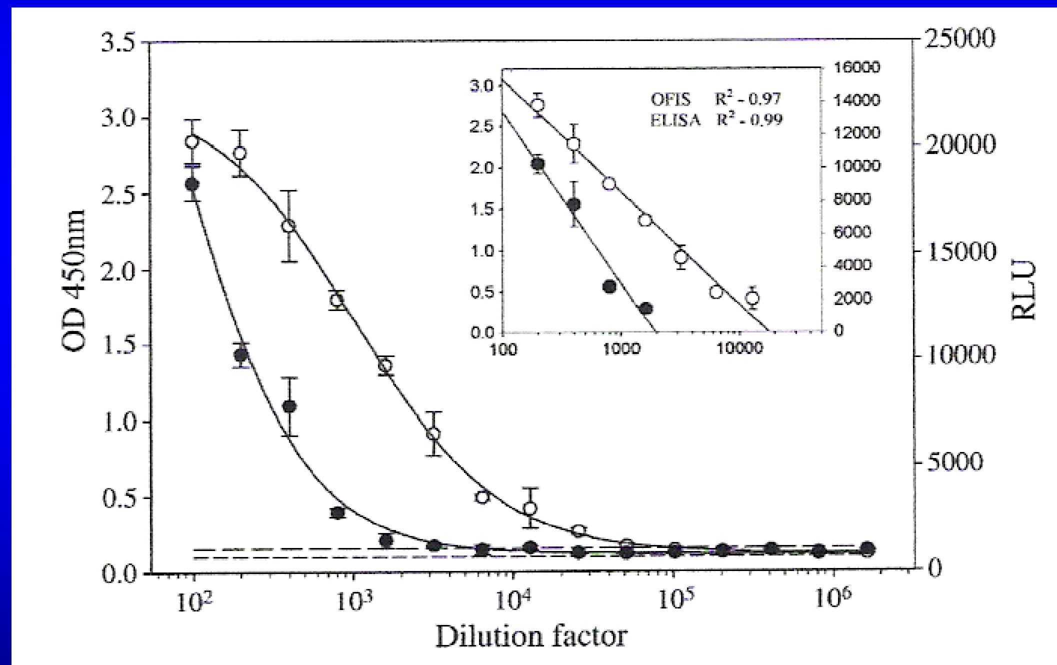

IgG Vaccinated sheep- Smithburn
ELISA PP value
0 20 40 60 80 100 120 140
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 20 28 34
Days post vaccination


IgM Vaccinated sheep- Smithburn
ELISA PP value
0 50 100 150 200 250 300
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 20 28 34
Days post vaccination

OPTICAL FIBER IMMUNSENSOR (OFIS) = biosensor with detection by chemiluminescence (Sorbazo et al 2007)

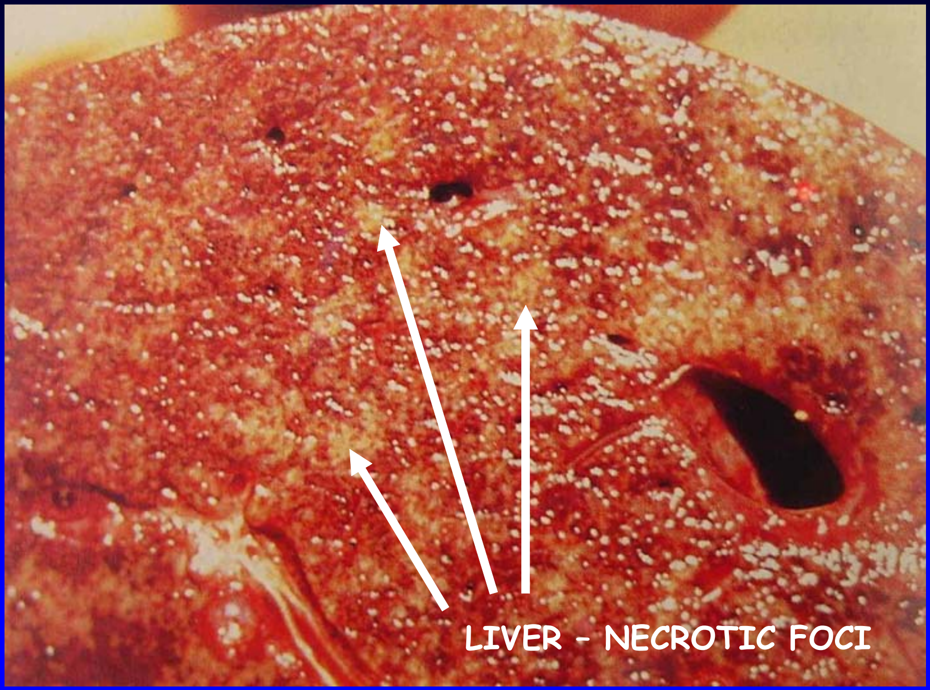
Human anti-RVF IgG - more sensitive and quicker than ELISA

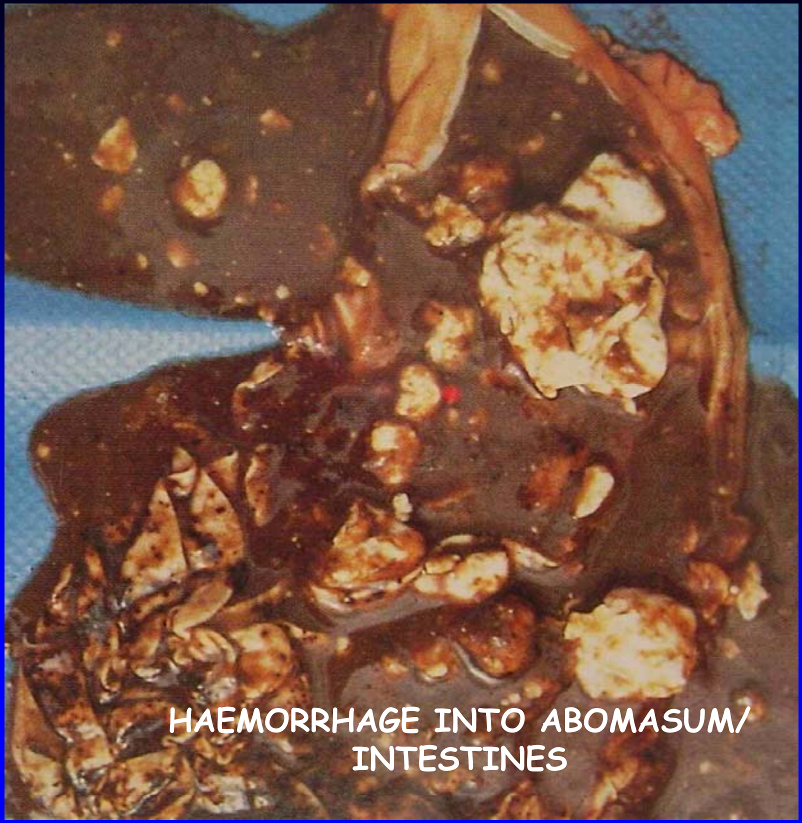


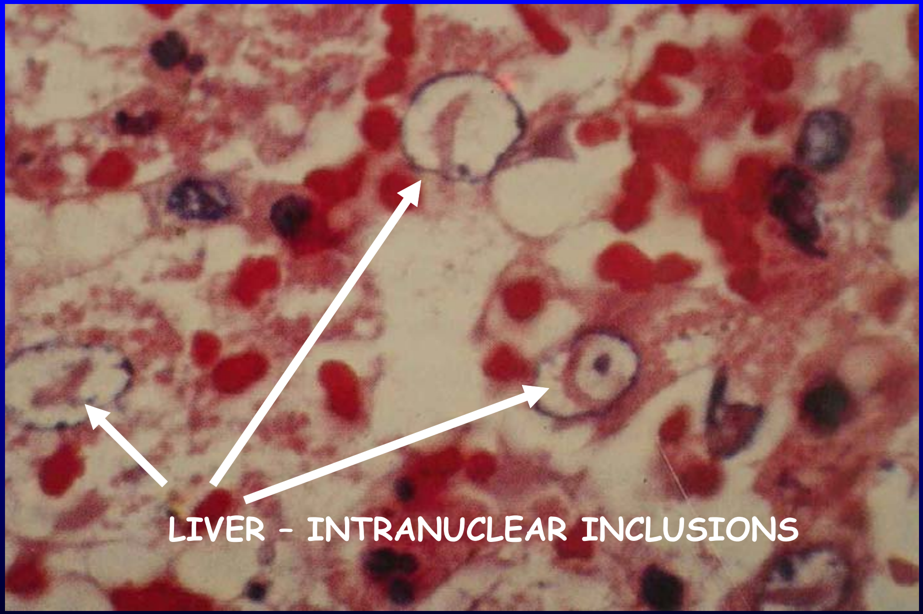


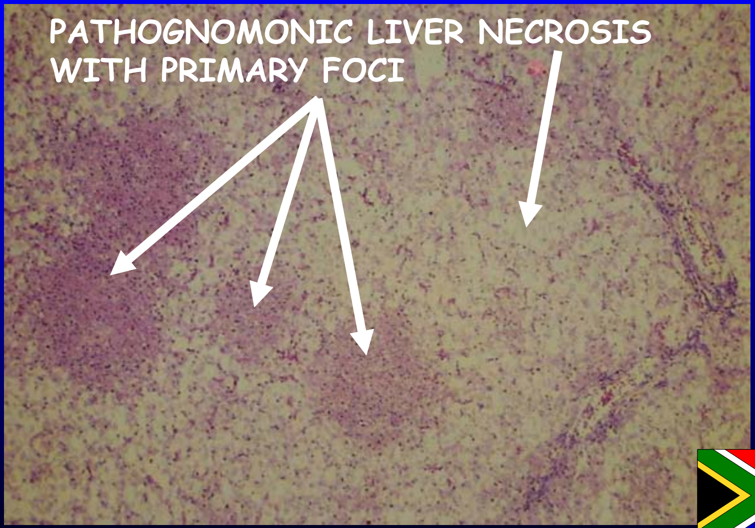
Aim: development of 'BIOPEN' for detection of viral antigens and antibodies

LESIONS IN LIVESTOCK (COETZER)









PREVENTION/CONTROL IN LIVESTOCK

- ROUTINE VACCINATION – ESPECIALLY EXOTIC BREEDS - WEANERS
- RAINFALL RSSD PREDICTIONS TO DRIVE COST-EFFECTIVE VACCINATION STRATEGIES???
- SMITHBURN MLVV – LIFELONG IMMUNITY BUT ONLY PARTIALLY ATTENUATED – SOME ABORTIONS – THEREFORE VACCINATE WEANERS – ANNUALLY
- INACTIVATED VACCINE EXPENSIVE AND REQUIRES 2 DOSES – PLUS BOOSTERS
- NEED FOR SAFE AND POTENT NEW VACCINES – HUMAN AND VETERINARY!