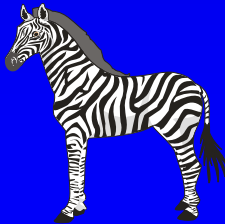
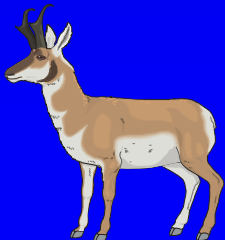
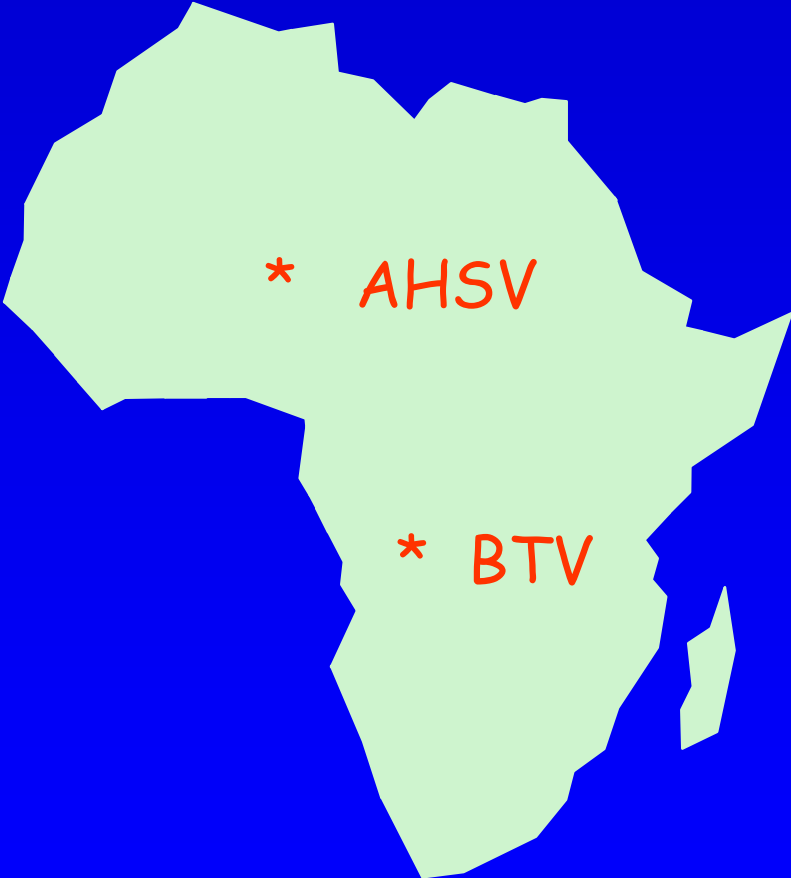


ORBIVIRUS DIAGNOSIS  
IN AN  
ENDEMIC  
COUNTRY

DR G.H. GERDES  
VIROLOGY STAFF  
OIE REF. LAB. STAFF

# GENESIS



# AHSV - SUSCEPTIBILITY

Resistant

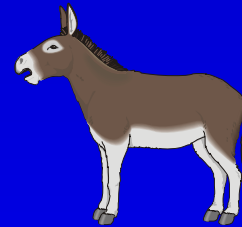
## Equids -

Horses

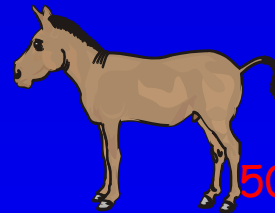
Mules

Donkeys

Zebra

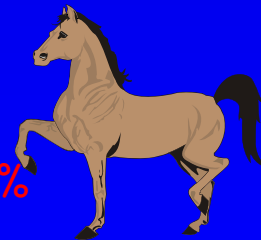


10%

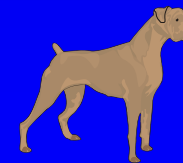


50-70%

## Canines



70-95%



1998 - AHSV 4

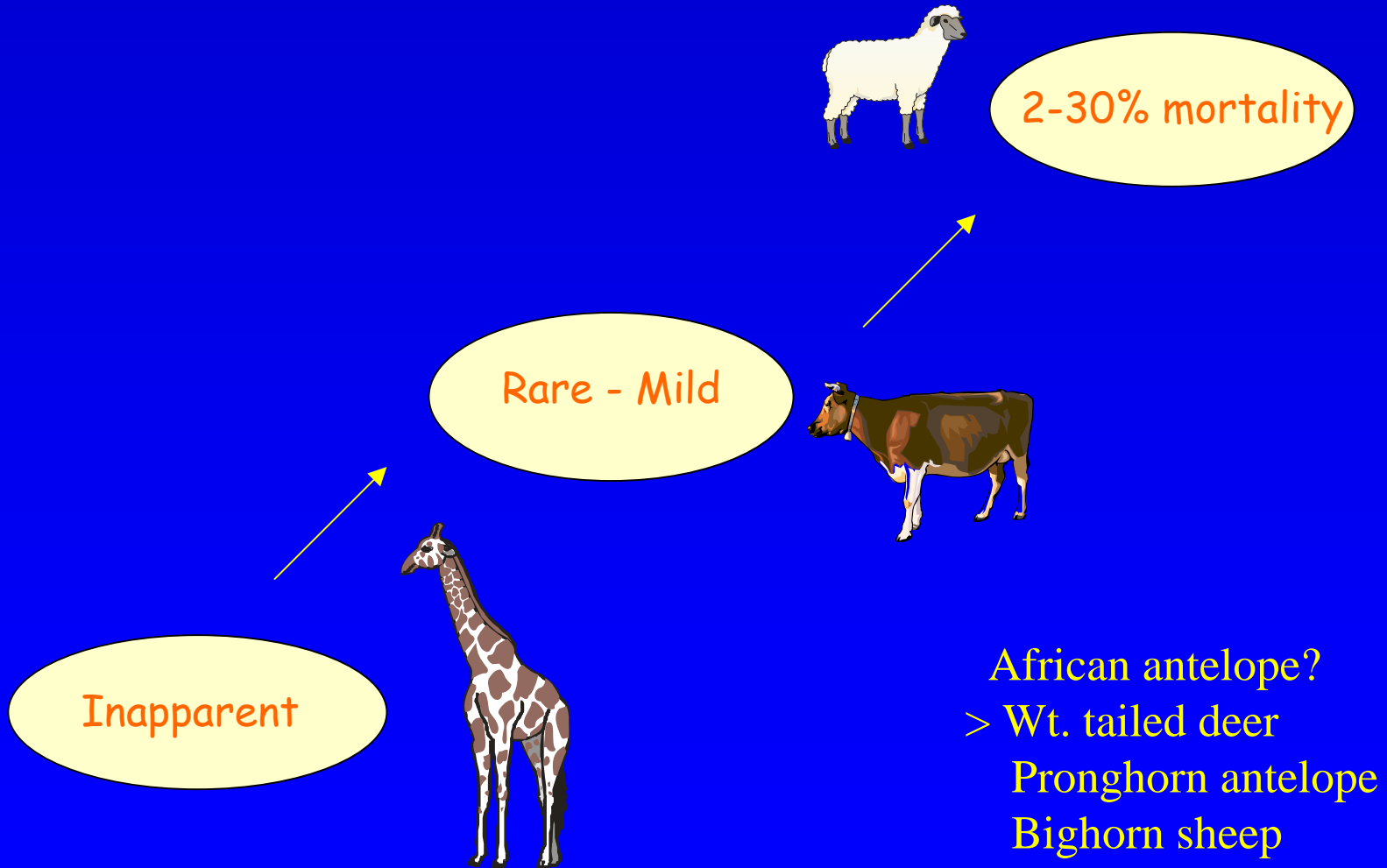
2006 - AHSV 6

Susceptible

Meat not the Midge

# BTV SUSCEPTIBILITY

All ruminants probably susceptible



# DIAGNOSTIC PROBLEM

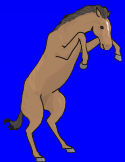
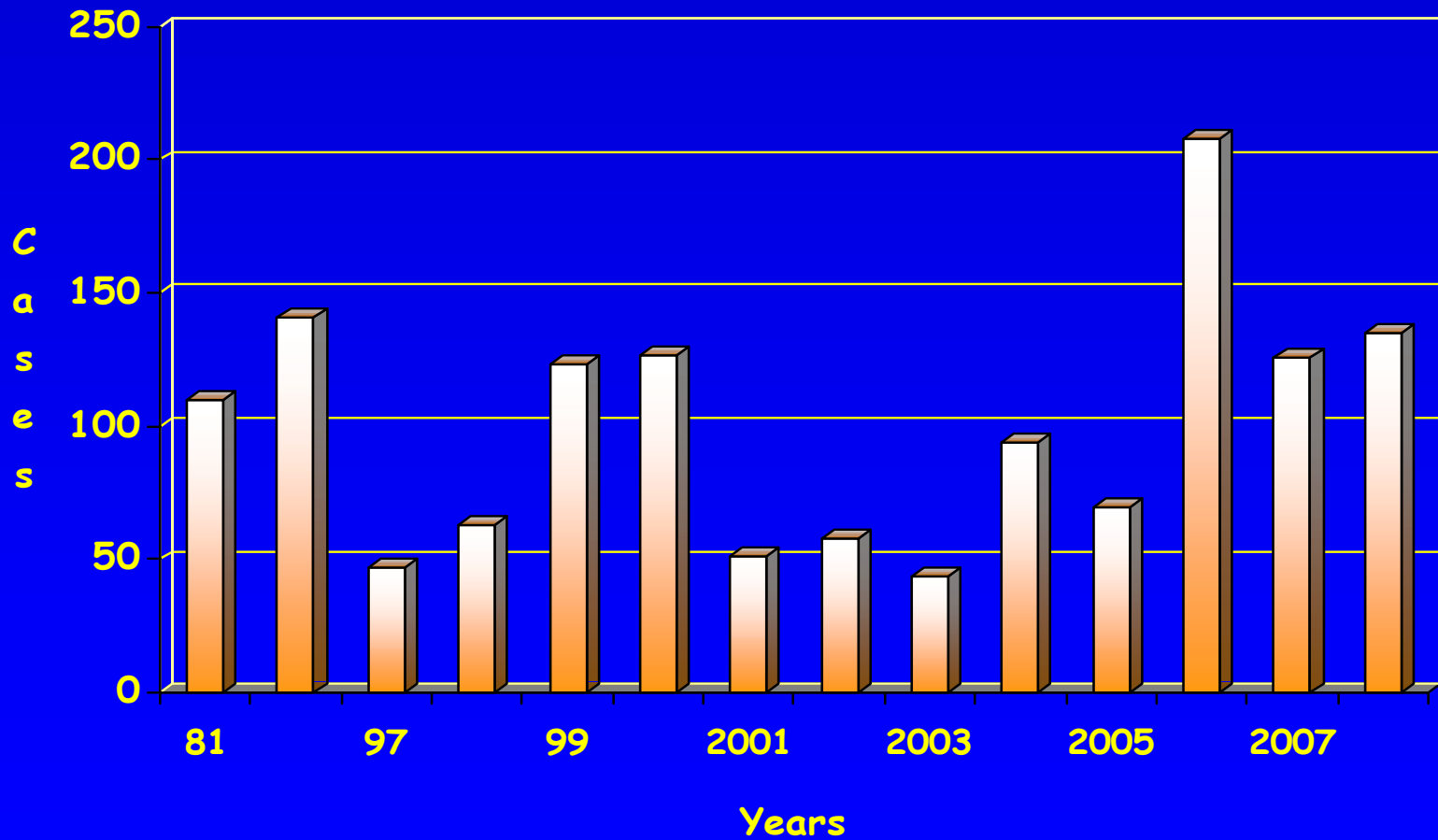
- a. Endemic
- multiple serotypes
  - related orbiviruses
    - EHDV
    - EEV

b. Diagnosis of a recent infection

- PCR X
- Isolation (X)
- A/bodies - IgG
  - IgM

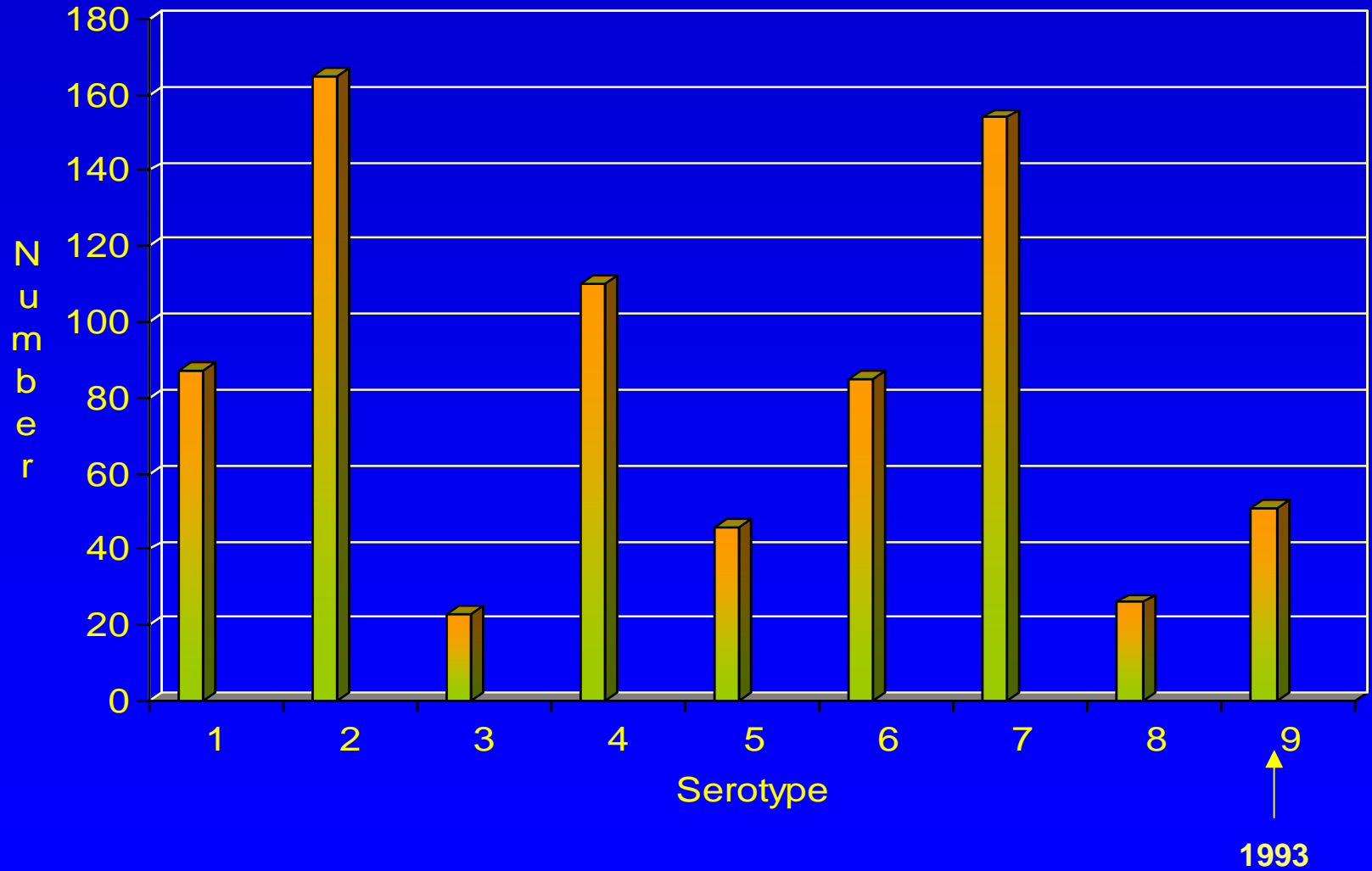
# AHS - CASE TOTALS

1981, 1996 - 2008



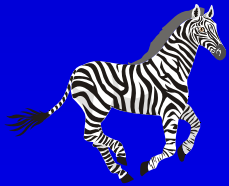
# AHS - SEROTYPES

1981 - 2008



# ROLE OF THE ZEBRA

- Was dominant equid (only)
- now confined to parks and farms
- Long viraemia 40d (blood) – 48d (spleen)
- Effectively circulates all 9 serotypes



S. West

N. East

6 Cape MT. Zebra

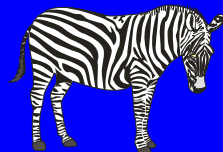
123 Burchells Zebra

Neg. x 9

5 mths – Neg x 9

10 – 12 mths – 100%

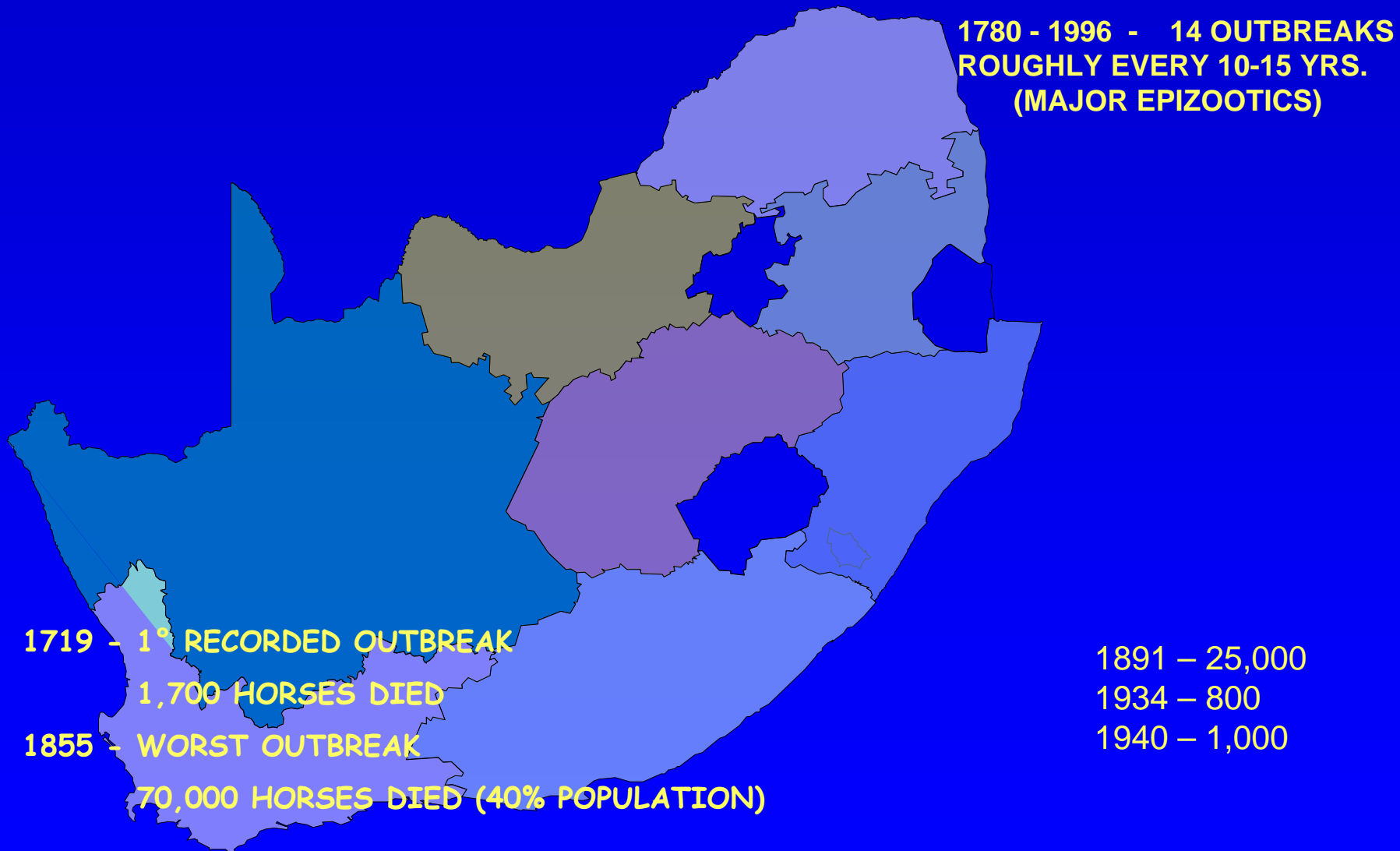
Pos.



= Virus Pool



# HISTORICAL - OUTBREAKS

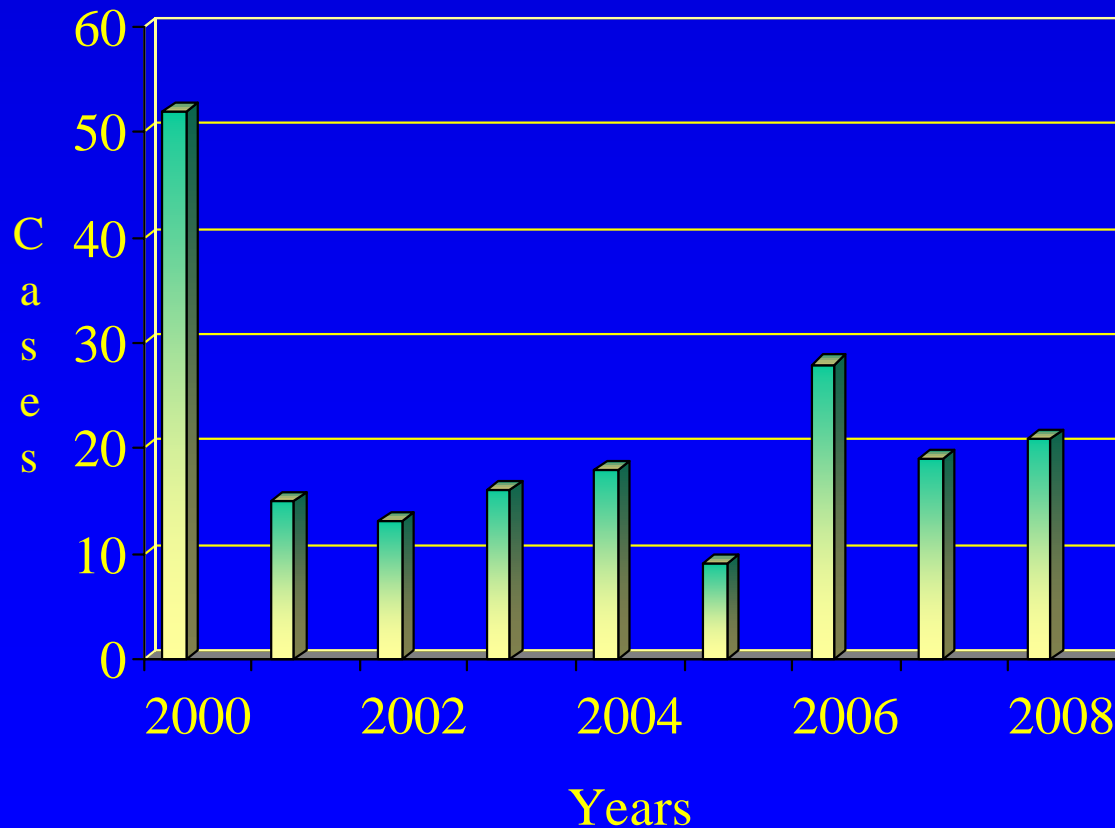


# BT - CASE TOTALS

2000 - 2008

Inapparent : Under Reported

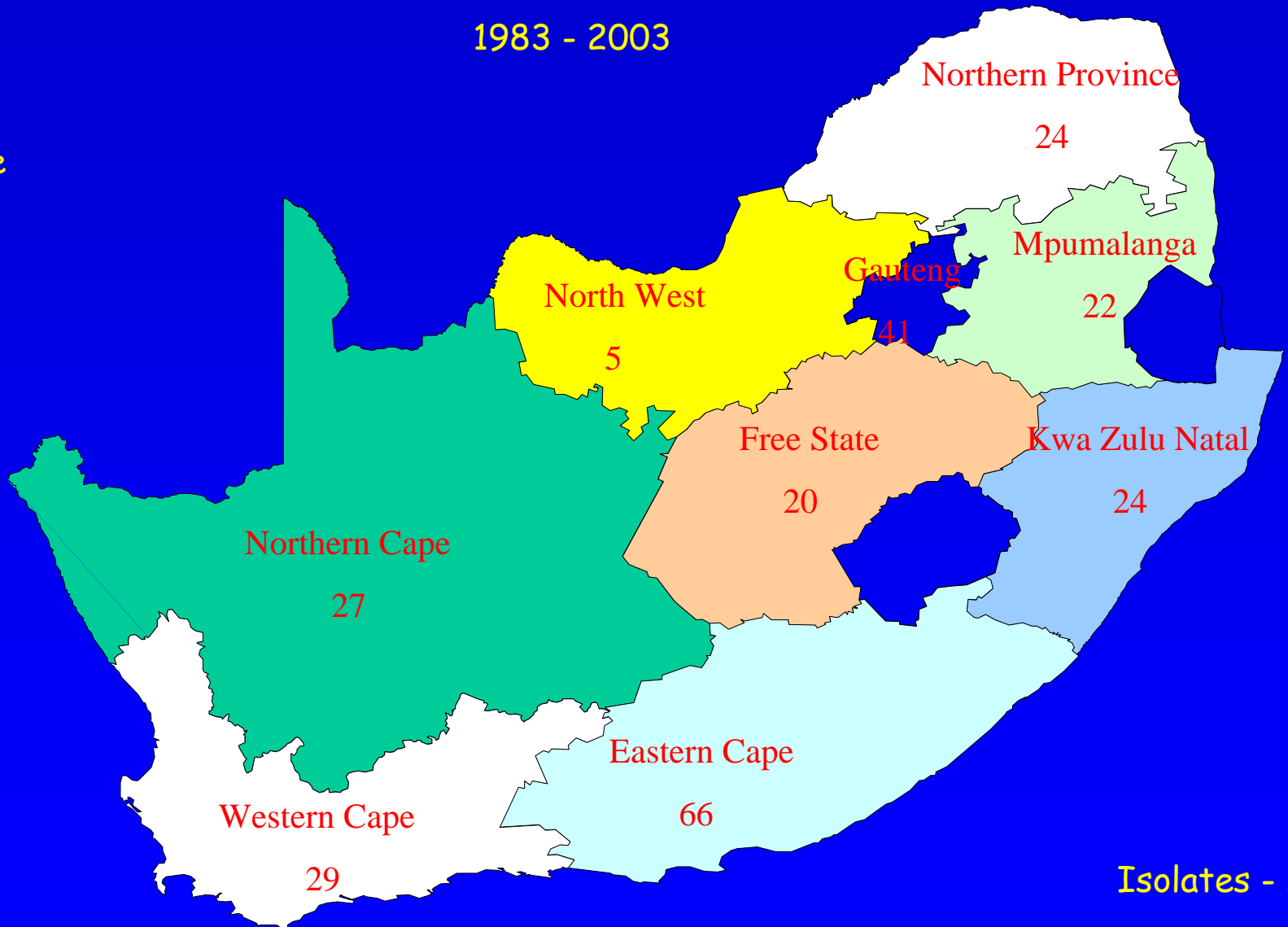
Notifiable - Animal Diseases Act. 1984



# PROVINCIAL BT ISOLATION TOTALS

1983 - 2003

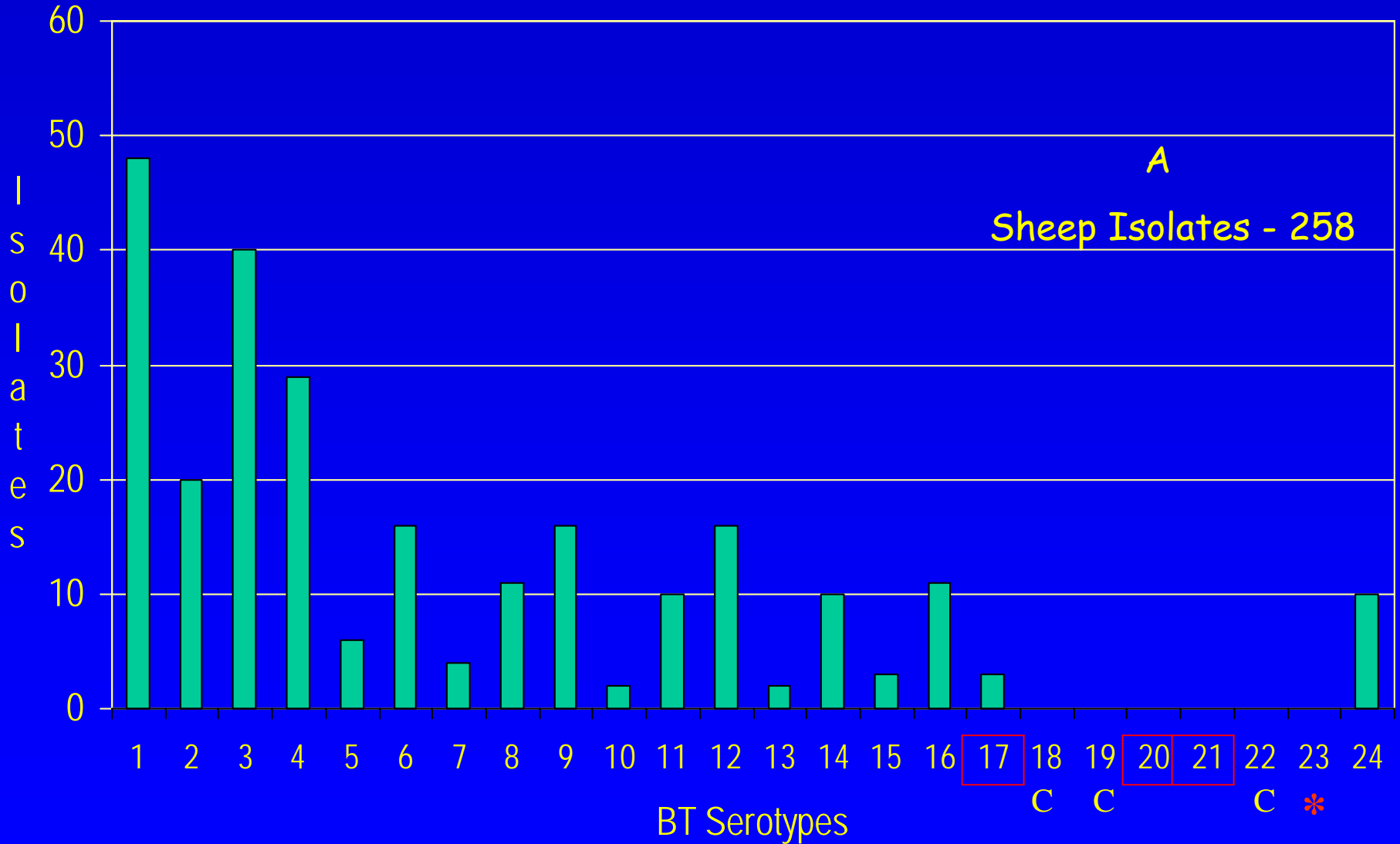
< game  
> bovine



Isolates - 258

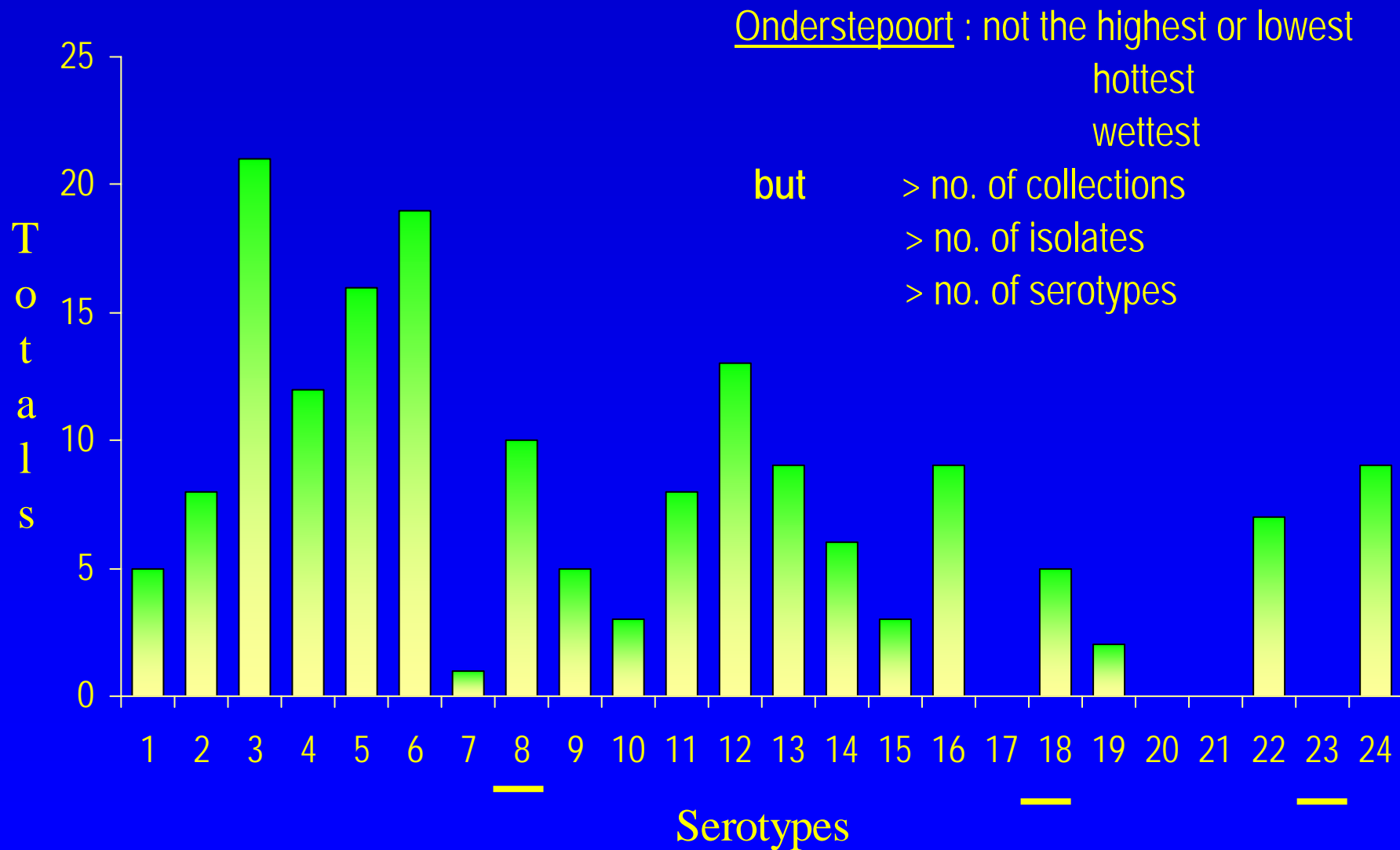
# BTV SEROTYPE TOTALS

1983 - 2003

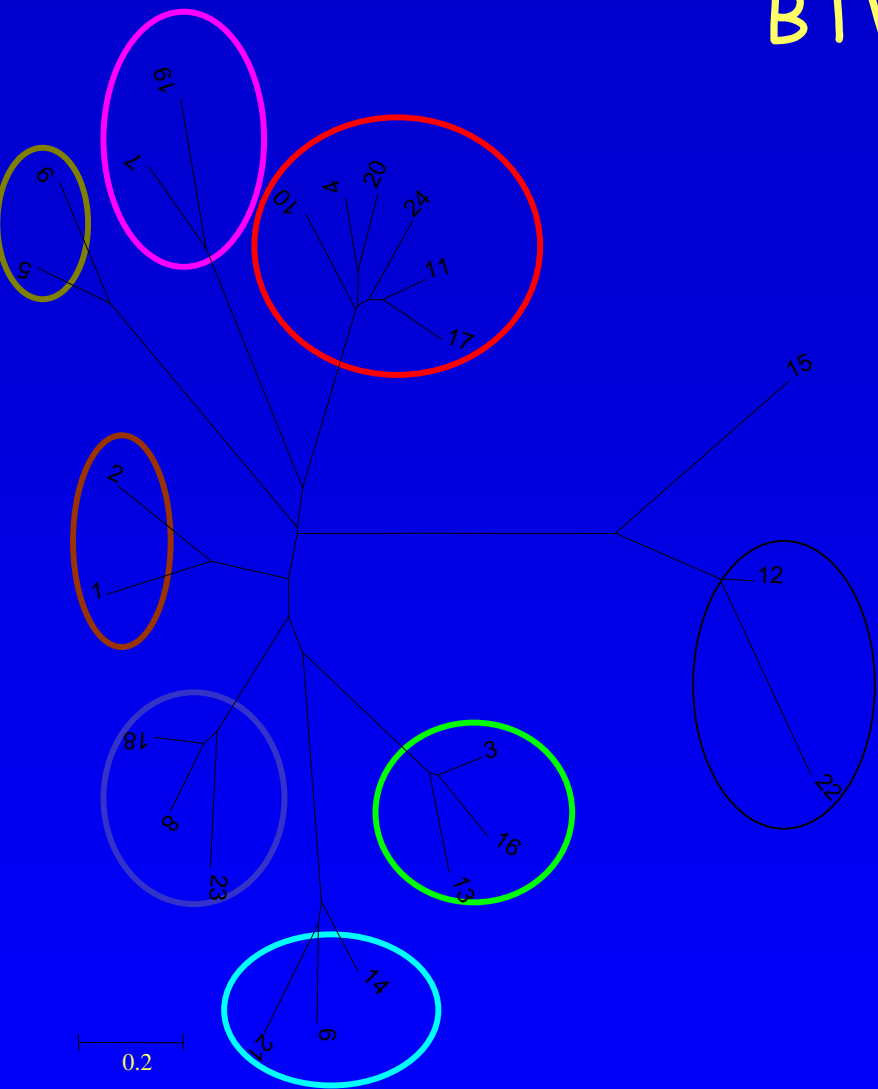


# CULICOIDES SURVEY

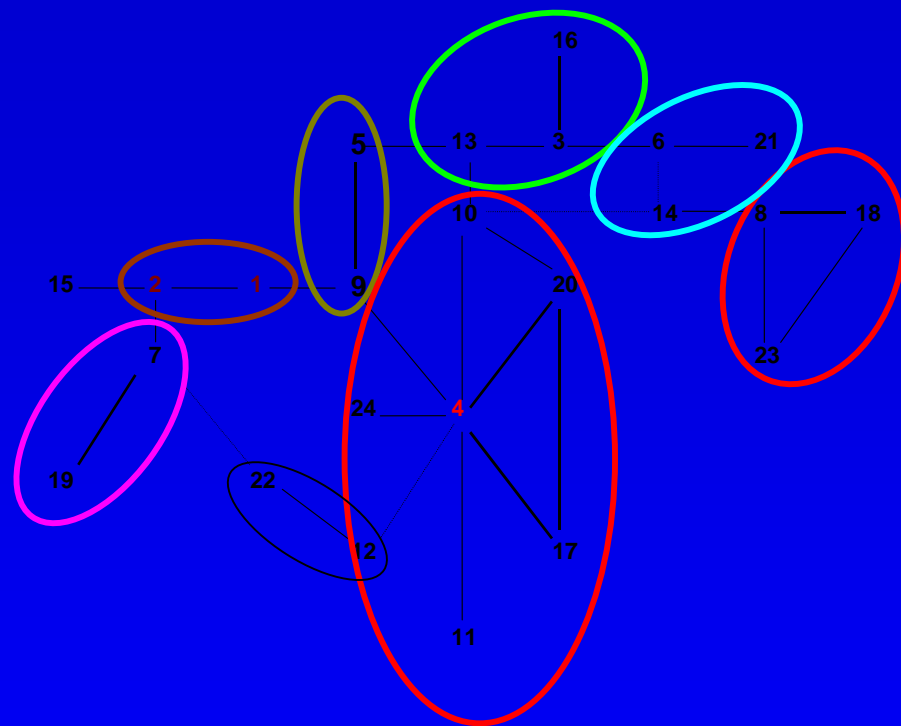
1978 - 1985



# BTV VP2



Phylogenetic relationship

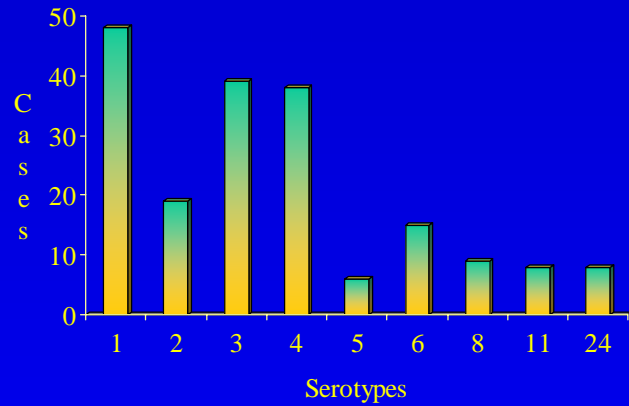


Serological relationship  
(in vitro)

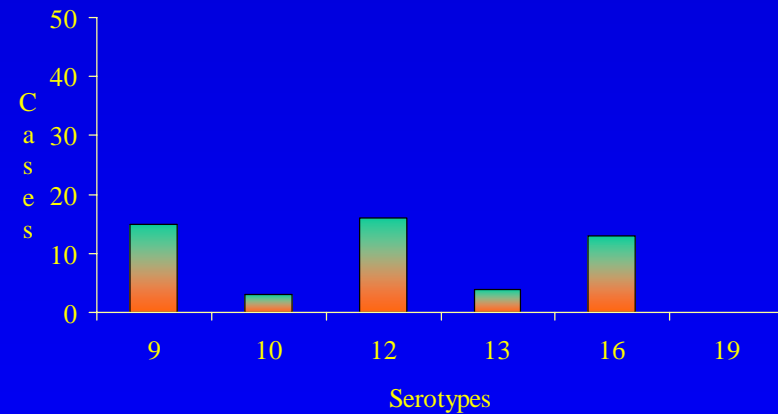
# SEASONAL SEROTYPES

1983 - 2003

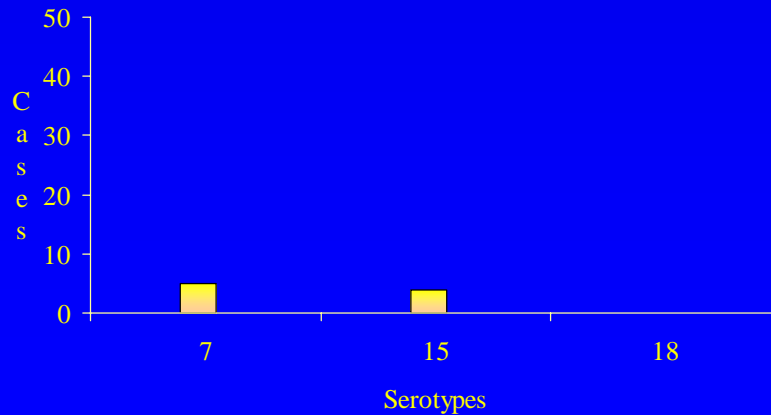
## HIGH EPIDEMIC POTENTIAL



## EVERY SEASON LOWER LEVELS



## LOW EPIDEMIC POTENTIAL



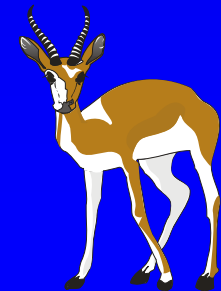
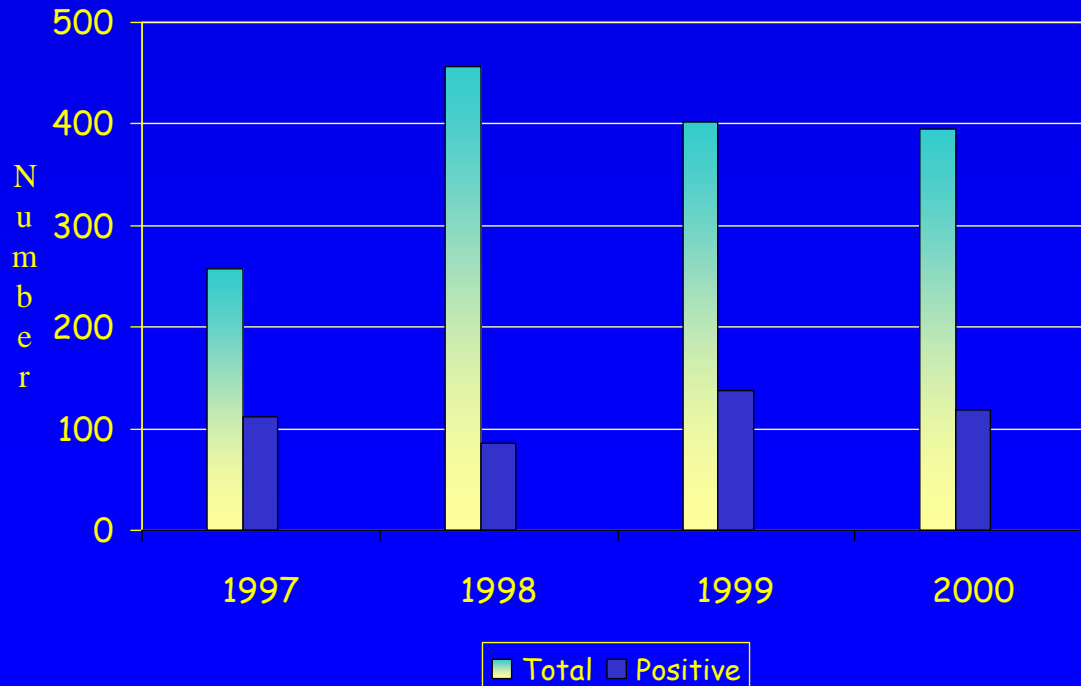
# BLUETONGUE IN GAME

Dec. < role as maintenance/amplifying host  
(23 official protected areas)

A - Export data

B - Survey data

A: In 4 yrs exported - 1,511 game 30% BT Pos.



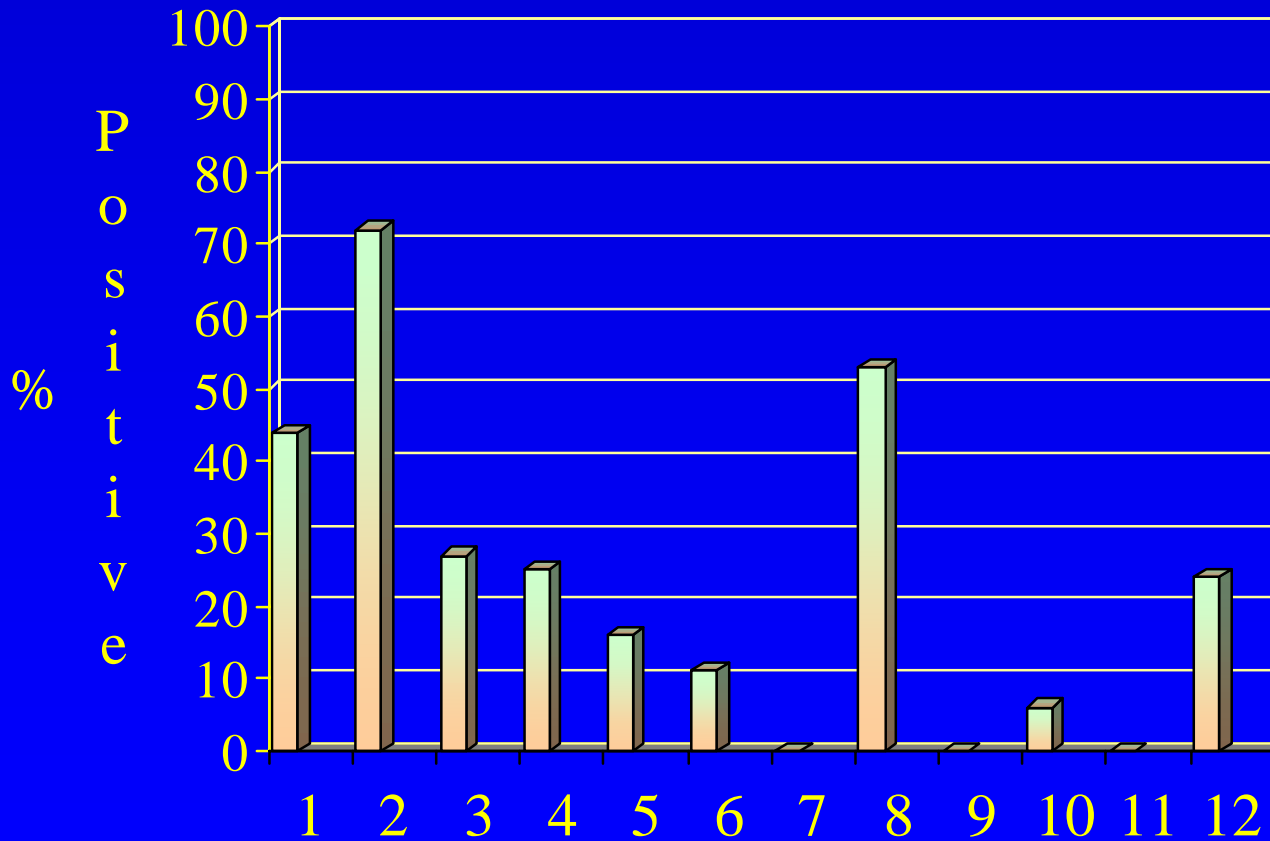


### B: Single survey 1997

- 4 vegetation types

- rainfall 280mm . 900mm

- \* 1. Blk w/beest
- \* 2. Bl w/beest
- 3. Red h/beest
- 4. Bl/buck
- 5. S/buck
- 6. Impala
- 7. G/buck
- \* 8. Buffalo
- 9. Kudu
- 10. Eland
- 11. B/buck
- 12. Giraffe



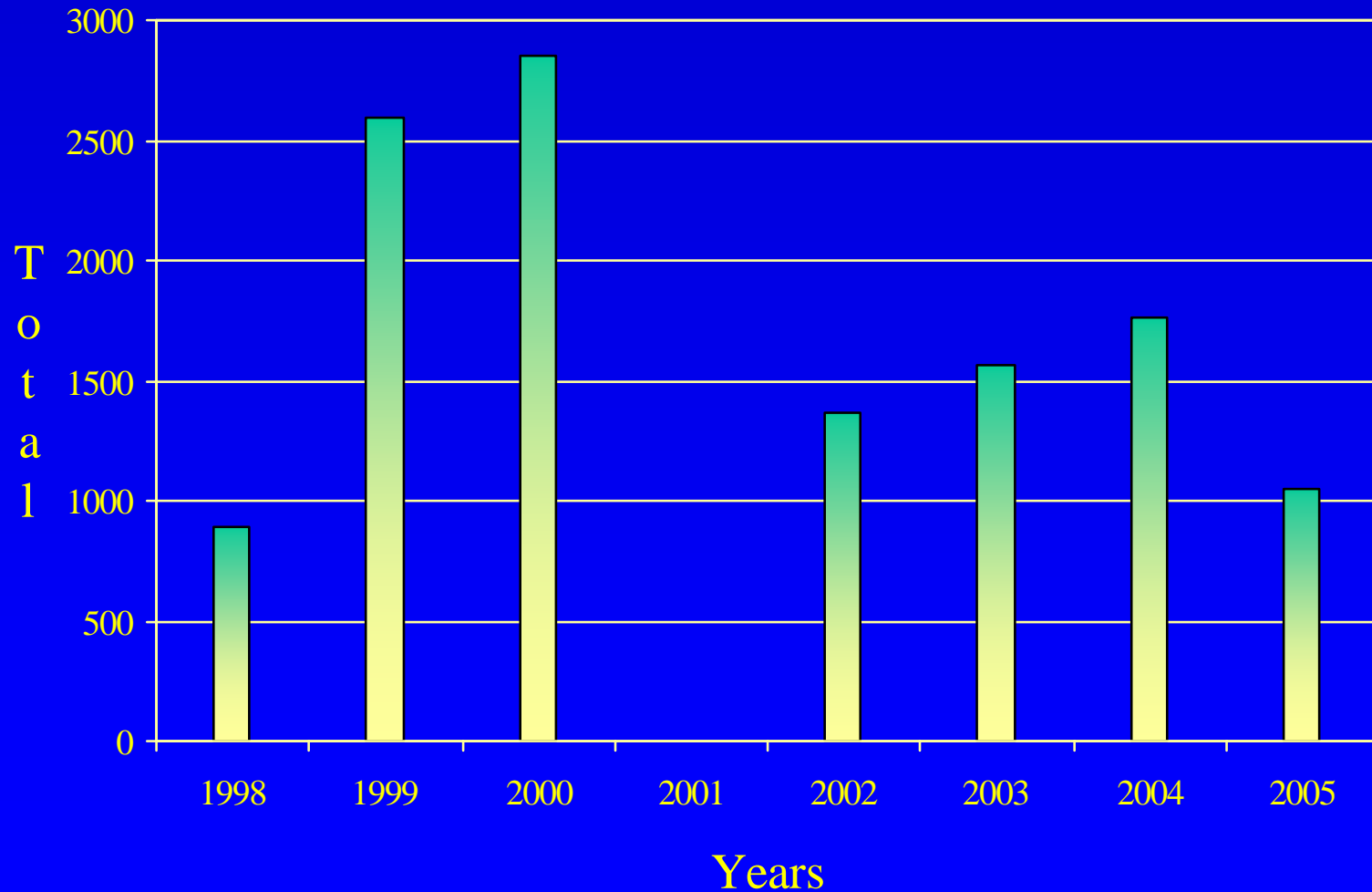
(Barnard 1997)

Game

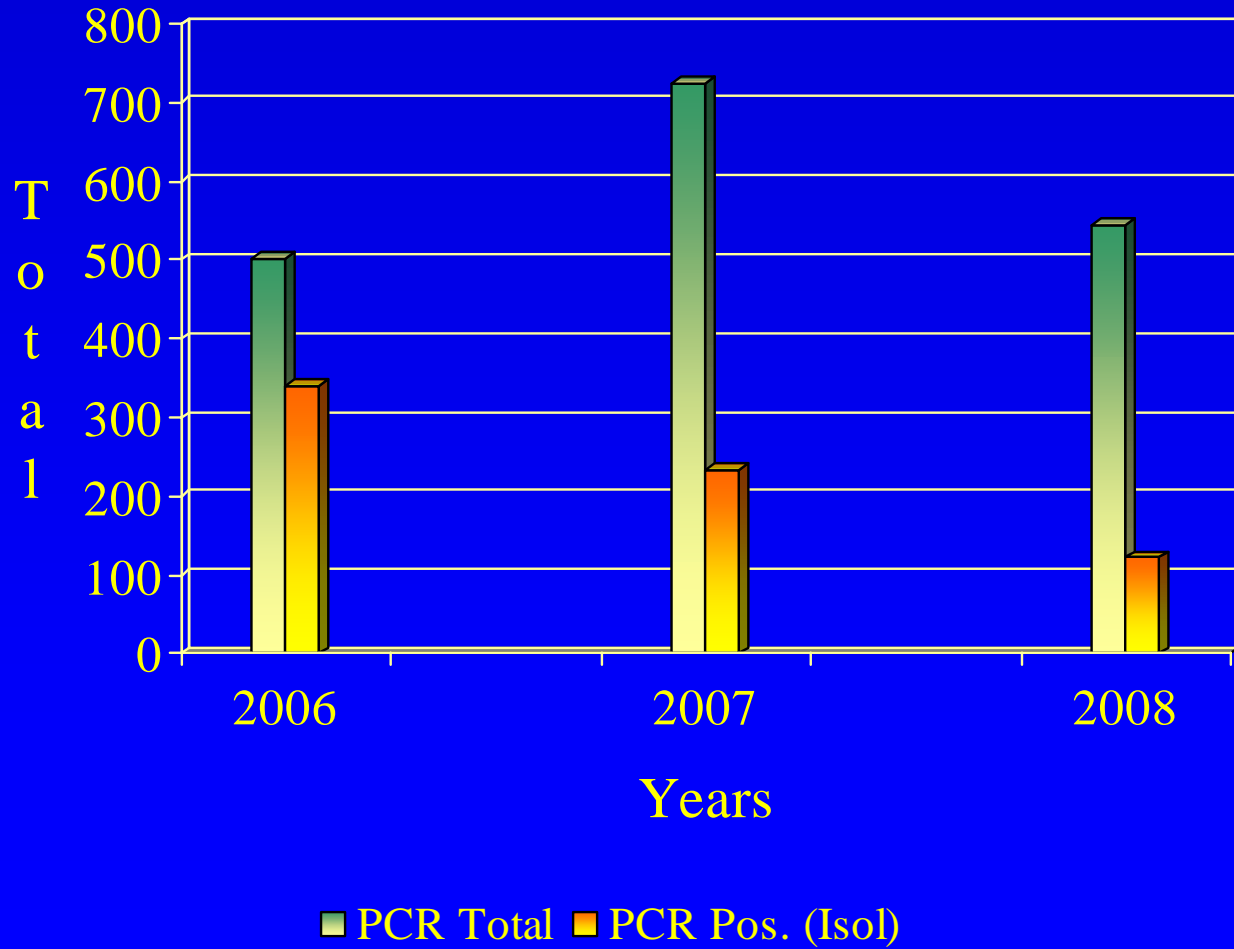
# BTV DIAGNOSTICS

## EMBRYO EXPORTS

Isolation (a)

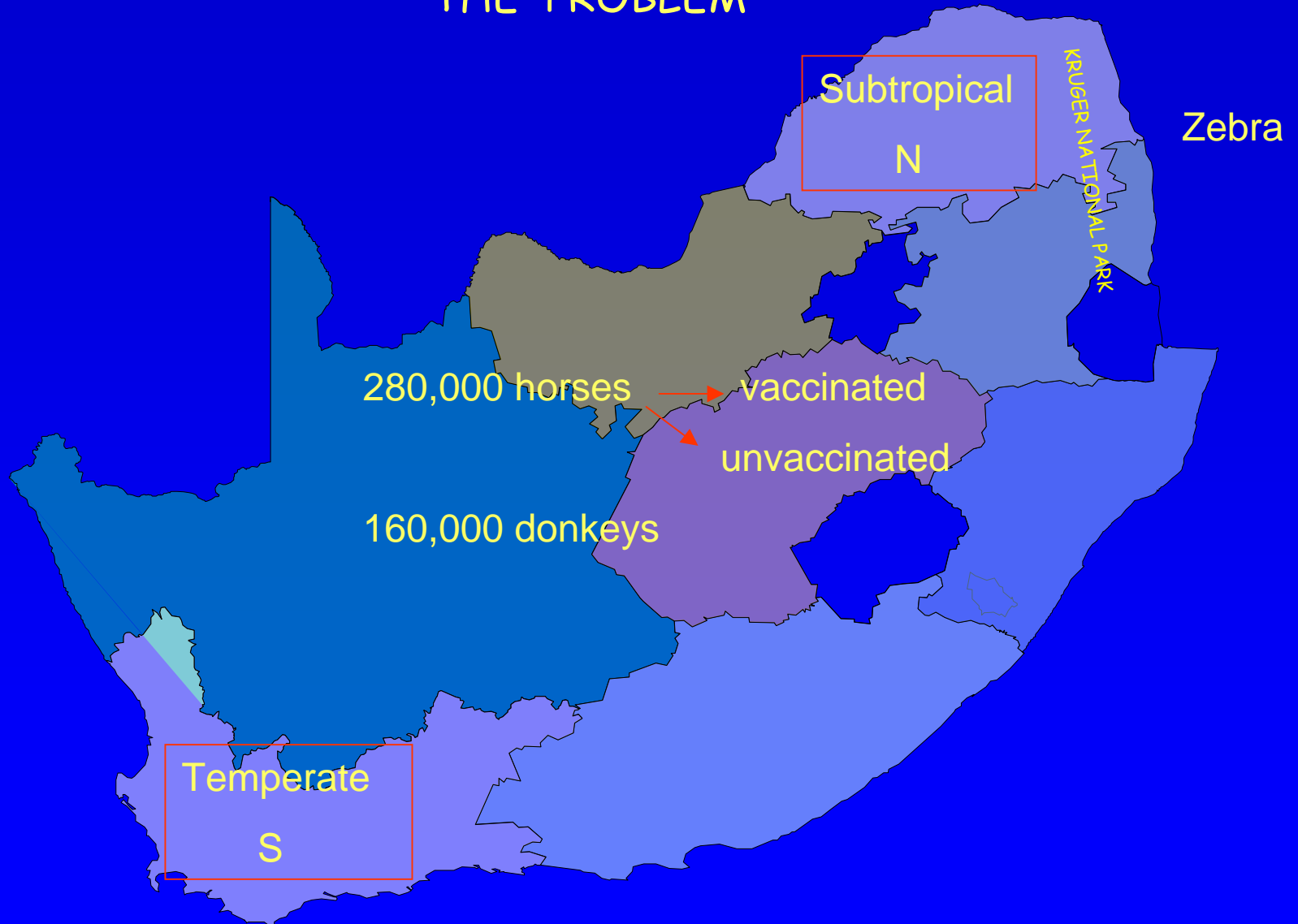


# PCR (b)



# AHSV DIAGNOSTICS

## THE PROBLEM

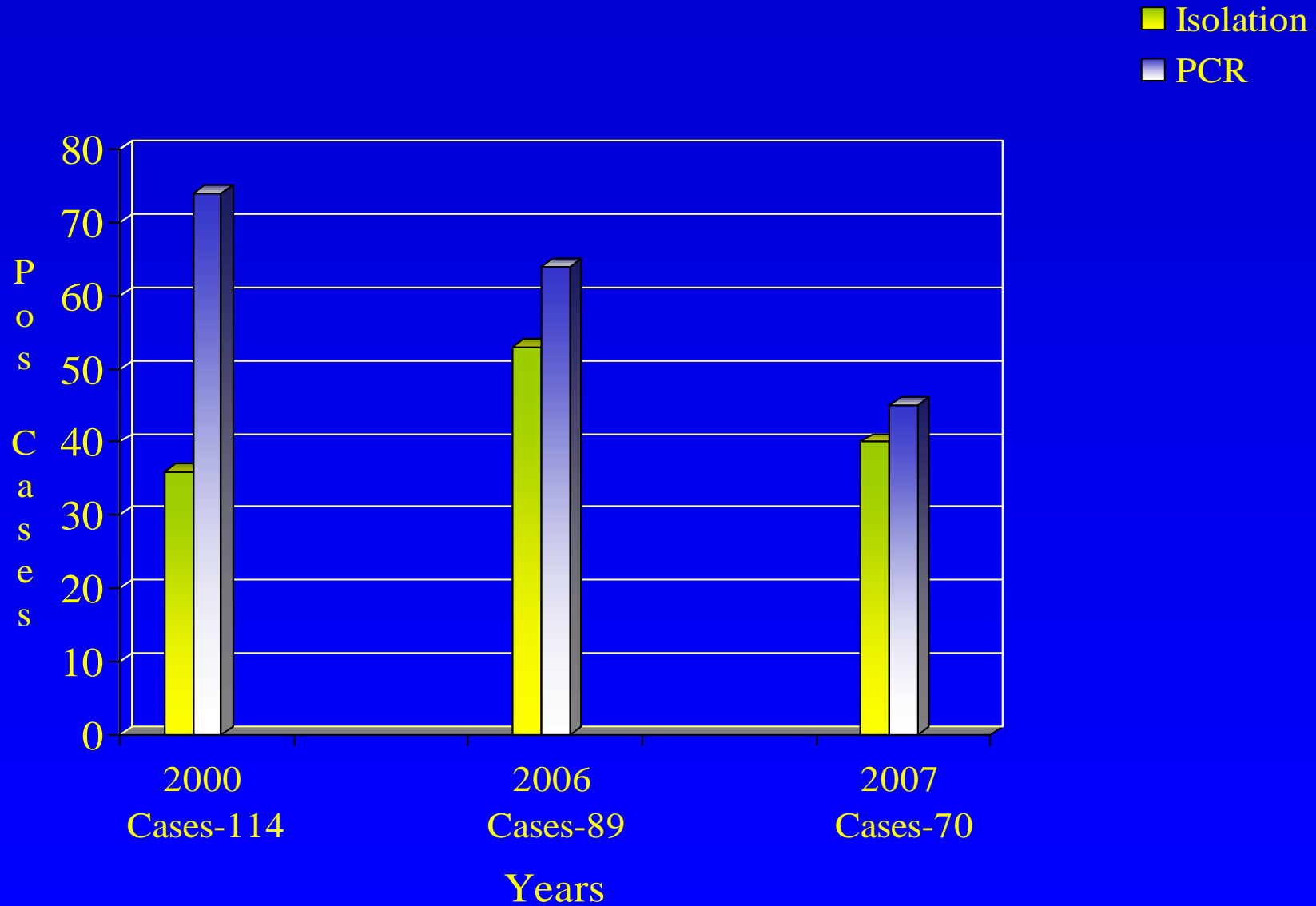


# RT. P.C.R.

- Not based on multiple sequence data - research tool (NS 2)
- Detects all 9 serotypes
- Does not detect EEV
- Cannot separate field : vaccine
- Remains positive > 3 months
- Require vaccination history
- 0,005 pfu of AHSV (sensitivity) - hemi-nested
- Sequence amplicon to exclude false positives.



# FIELD DATA



# ISOLATION

EDTA ; HEPARIN ; SERUM (clot)

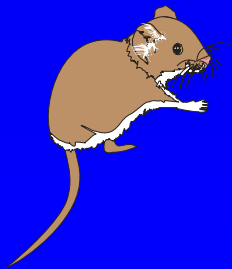
*Spleen*      &      *Lung*



90%



10%



- i/c infant mice -----> select for AHSV
- tissue culture -----> select for EEV

# ISOLATION

(cont)

- \* No complicating antibodies

<u>AHS</u>	mice	d 3 - 5
	TC	d 7+

- \* Other

<u>AHS</u>	mice	d 7 -10 or #2
	TC	#2



# DUAL INFECTION

(cont.)

Spleen - PCR positive AHSV  
- isolate EEV



Filters/catabolizes RBC

AHSV bound to RBC (wash & lyse to isolate)

Spleen - virus recepticle



SPRING : AUTUMN

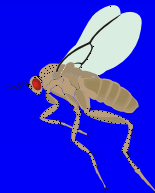
virus circulation



# AG - C - ELISA

## AHS

- Can't do blood
- Spleen > 60% +ve: Isolation
- Useful to screen TC before typing (EEV)
- Midge?



# HUMORAL A/BODY RESPONSE

B cells - plasma cells - 3,000 → 30,000 a/b. molec./cell/sec.

- single specificity
- total response is heterogeneous
- responds by producing

IgA

IgG

IgD

IgM

AgE

- IgM serum half life - 5 days
- IgG serum half life - 25 days

(human data)

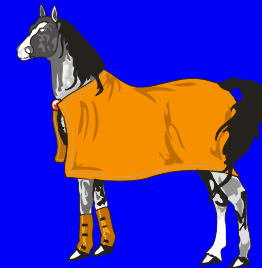
# IgM & IgG

## IgM

- first a/body
- large, enters interstitial fluid slowly (not placenta)
- effective against micro organisms
- pentamer - mops up 5 x organisms

## IgG

- most prevalent a/body in blood (also placenta)
- produced later in response
- monomer
- stimulating dose low = IgM  
high = IgG
- triggers cf but less effective



# ANTIBODIES

(cont.)

## Primary & Secondary Stimulation

Antigen A

IgM	d 3 - d 15	] Bell curve arbitrary unit 1
IgG	d 7 - d 28	

2<sup>nd</sup> Antigen A

IgM d 28 - d 40 unit 1

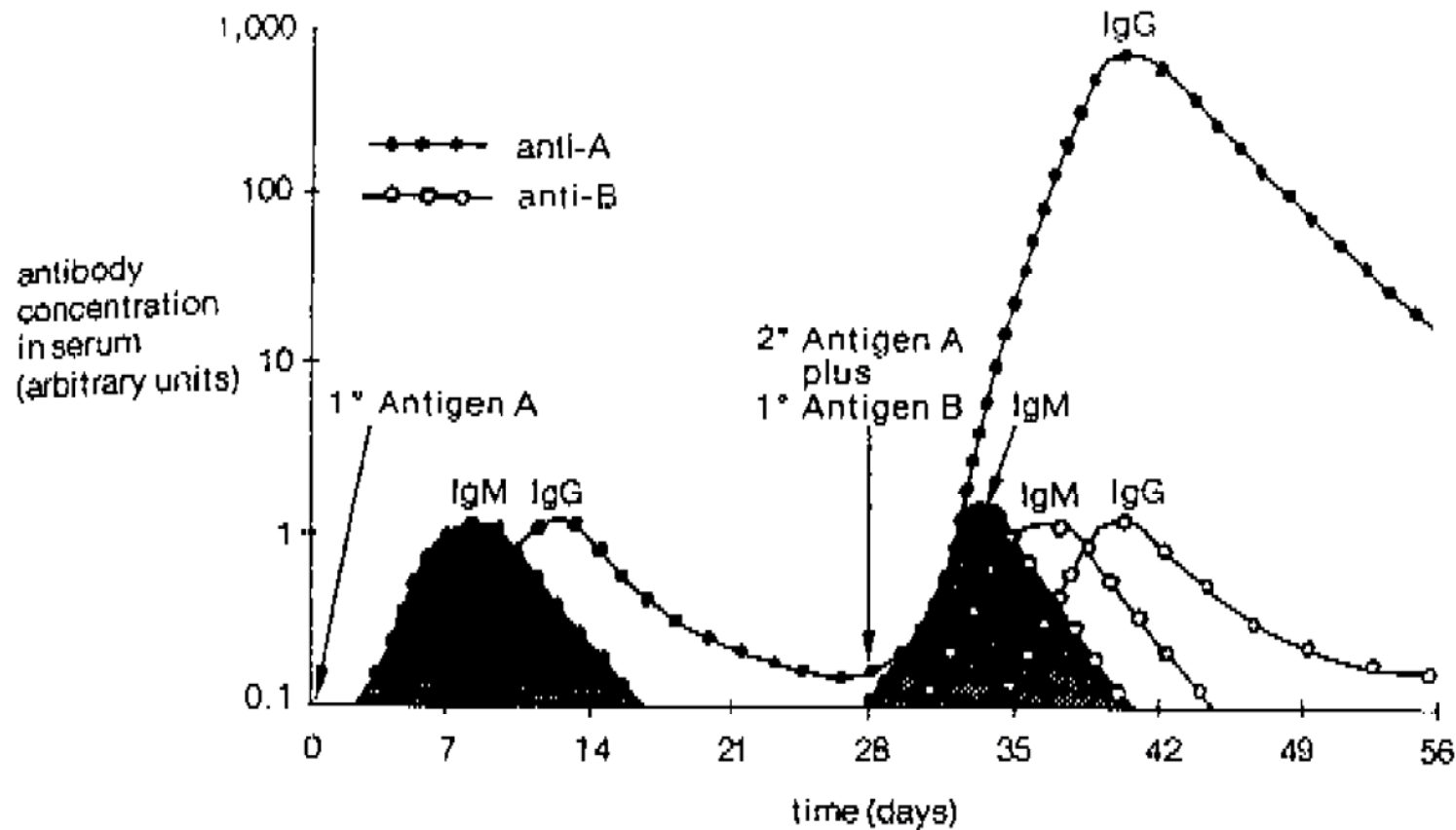
IgG d 28 - d > 56 unit 1,000 (anamnestic response)

Antigen B Ditto Antigen A

Challenge d 28 - immediate response A

- 3 - 7 d lag for B

WHAT IF THESE ARE AHSV/EEV SEROTYPES (Ag A & B)



**Figure 1-31** Kinetics of IgM and IgG appearance in the serum following primary and secondary immunizations. The secondary response to Antigen A demonstrates that the IgM response is slightly more rapid and intense than the primary IgG response. However, most of the secondary response is of the IgG class.

# FIELD DATA

## Serology/Isolation

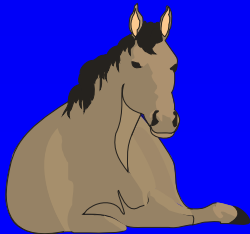
AHS Cft	EE Cft	Isolation
Neg	Neg	Neg
1:128	1:16	Neg
1:192	1:16	AHSV 6
1:16	1:192	AHSV 7
1:24	1:64	AHSV 7
1:64	1:48	AHSV 5
1:4	Neg	EEV Kaalplaas

# TESTS

- \* Complement fixation - old fashioned
  - labour intensive
  - overnight incubation
  - insensitive - 10 d. delay

## THIS IS ALL WE HAVE

- \* i-Elisa
  - recombinant antigen
  - well validated
  - first generation Elisa
  - stable in side x side testing
  - not useful - endemic
    - vaccination







THANK YOU