

Blue tongue virus in Kenya

Insights from Isiolo and progress on molecular typing and virus isolation

Nicholas Svitek
PALE-Blu Blue Tongue Virus Consortium Meeting
Giulianova, Italy
26 September 2019







AVIS N° : A N° 0134121

TE 2119118
 HEURE 14h04
 MATRICULE 002556A
 MARQUE/COULEUR 121-A bleu

ZONE A
 CIRCUIT S9

Cet avis a été dressé pour

Stationnement hors zone d'abonnement الووقوف خارج منطقة الانخراط ☐ Stationnement sans ticket الووقوف دون تذكرة ☒
 Non respect des places réservées عدم احترام أماكن الركن المخصصة ☐ Abonnement expiré انتهاء صلاحية بطاقة الانخراط ☐
 Délai de 10 minutes sans ticket dépassé تجاوز مدة الوقوف المؤدى بأكثر من 10 دقائق ☐

Informations pour libérer votre voiture معلومات لتحرير سيارتكم

1- Payer 30 DH pour non respect des règles de stationnement dans la zone bleue 1 - أداء مبلغ 30 درهم في العداد لعدم التزامكم بشروط الوقوف في المنطقة الزرقاء

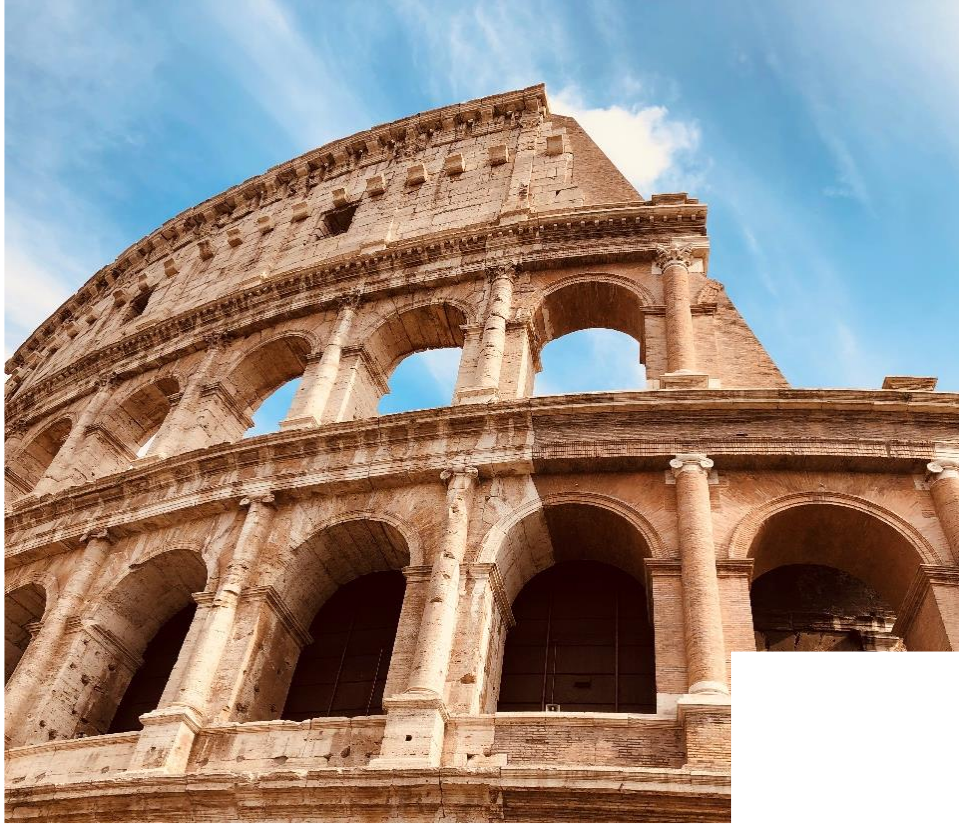
Contactez le 05 22 99 35 80 05 22 24 67 68 063825609

Permanence

الخدمة من 08 صباحا طيلة الأسبوع ما عدا الأحد والأعياد
 Service de 8h du matin toute la semaine sauf dimanche et jours fériés

2 - الاتصال
 3 - المداومة





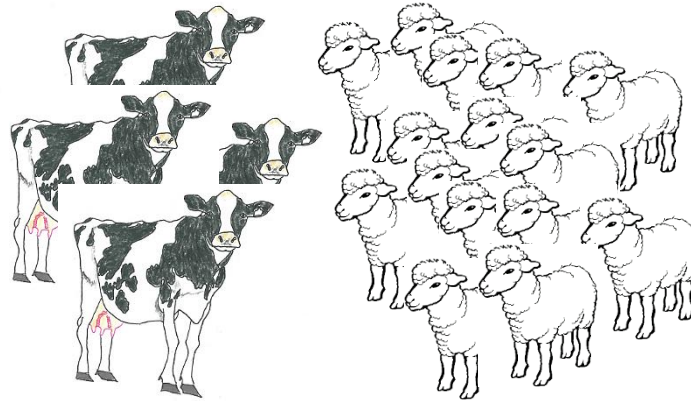


Bluetongue virus (BTV)

Our Involvement in the Project

WP2: Identify and characterize BTV strains circulating in ‘source’ regions that represent potential threats to Europe

WP2: Work Plan / Study Design



Field sampling during
reported **outbreaks** and in
known **high prevalence**
areas

- Serology [ID Screen® Bluetongue Competition (anti-VP7)]
- Molecular epidemiology by PCR (segment 10)/serotype specific qPCR (VP2 gene)
- Sequencing of field samples or
- Genomic characterization of virus isolates



Isolation of virus on
BSR or KC cells by
plaque purification

BTV in Kenya

- ✓ **1978** : 19 different BTV serotypes found in sentinel cattle near Nairobi
 - *Rainfall had no obvious effect on the serum conversion rates.*
- ✓ **2013** : In Busia (Western Kenya) 94.2% anti-BTV in cattle (n=455)
 - (single: BTV-7,-15,-16,-19, dual: BTV-1&-12, BTV-3&24, BTV-22&24).
 - *Rainfall had no obvious effect on the serum conversion rates.*

BTV Outbreak in Kenya-2018



Bluetongue,
Kenya



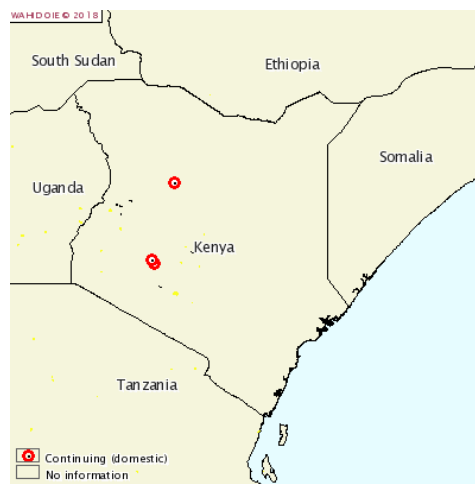
Information received on 20/07/2018 from Dr Obadiah Nyaga Njagi, Director of Veterinary Services, Directorate of Veterinary Services, State Department of Livestock, Ministry of Agriculture, Livestock and Fisheries, NAIROBI, Kenya

Summary

Report type	Immediate notification
Date of start of the event	20/06/2018
Date of confirmation of the event	25/06/2018
Report date	20/07/2018
Date submitted to OIE	20/07/2018
Reason for notification	Recurrence of a listed disease
Date of previous occurrence	1998
Manifestation of disease	Clinical disease
Causal agent	Bluetongue virus
Serotype	Not typed
Nature of diagnosis	Laboratory (basic)
This event pertains to	a defined zone within the country
Related reports	Immediate notification (20/07/2018) Follow-up report No. 1 (06/08/2018) Follow-up report No. 2 (15/08/2018) Follow-up report No. 3 (28/08/2018)

New outbreaks (3)

Outbreak 1		Samburu Central, Samburu					
Date of start of the outbreak		20/06/2018					
Outbreak status		Continuing (or date resolved not provided)					
Epidemiological unit		Farm					
Affected animals		Species	Susceptible	Cases	Deaths	Killed and disposed of	Slaughtered
		Sheep	350	64	0	0	0
Outbreak 2		Gilgil, Nakuru					
Date of start of the outbreak		20/06/2018					
Outbreak status		Continuing (or date resolved not provided)					
Epidemiological unit		Farm					
Affected animals		Species	Susceptible	Cases	Deaths	Killed and disposed of	Slaughtered
		Sheep	100	10	5	0	0
Outbreak 3		Nakuru, Nakuru					
Date of start of the outbreak		20/06/2018					
Outbreak status		Continuing (or date resolved not provided)					
Epidemiological unit		Farm					
Affected animals		Species	Susceptible	Cases	Deaths	Killed and disposed of	Slaughtered
		Sheep	150	15	0	0	0



Summary:

of outbreak: **3**

Areas: **2** [**Samburu** and **Nakuru**]

Species: **sheep**

Number of cases: **89/600**

Deaths: **5**

Case fatality rate: **5.62%**

Summary of Years 1 & 2

Year 1

- Getting reagents & protocols
- Getting paper work/approvals
- Training at Pirbright
- Sampling in Machakos
- cELISA with Machakos samples
- Problem with qPCR assay

Year 2

- Finalized cELISA of Machakos samples
- Validated qPCR assay with new probes/primers
- Analyzed blood samples from Machakos by qPCR
- Analyzed Isiolo samples by cELISA & qPCR
- Isolated viruses from the field

BTV in Machakos County – Kapiti Plains

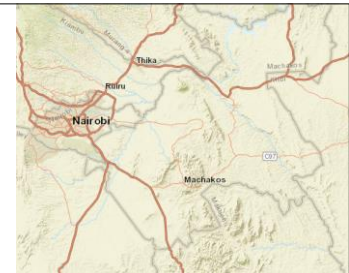


Date of sampling : **May 17-18, 2018**

Season: **towards the end of the long rainy season**

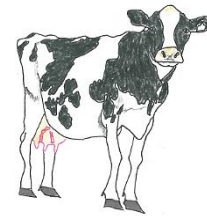
Location: **Kapiti Plains** [ILRI's research station in Machakos]

Coordinates: **1°38'01.2"S 37°08'51.2"E**



Sampling for BTV in Machakos County

$$n' = \frac{1}{1/n + 1/N}$$



12
herds

Dairy herd: **18/149**
Herd 1: **15/125**
Herd 2: **15/116**
Herd 3: **15/116**
Herd 4: **15/120**
Heifers 1: **15/129**
Heifers 2: **18/153**
Heifers 3: **14/119**
Steers 1: **13/108**
Steers 2: **15/125**
Steers 3: **15/129**
Steers 5: **14/120**

Cattle in Kapiti 2581 – adjusted n = **180**

Goats in Kapiti 306 – adjusted n = **121**

Sheep in Kapiti 1326 – adjusted n = **169**

Camel 37 [**~80**] in Kapiti – adjusted n = **31 [43]**



3
herds

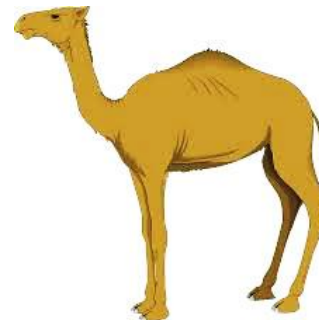
Herd 1: **females [milking]**
Herd 2: **females [dry]**
Herd 3: **males**



3
herds

Herd 1: **females [older]**
Herd 2: **females [younger]**
Herd 3: **males**

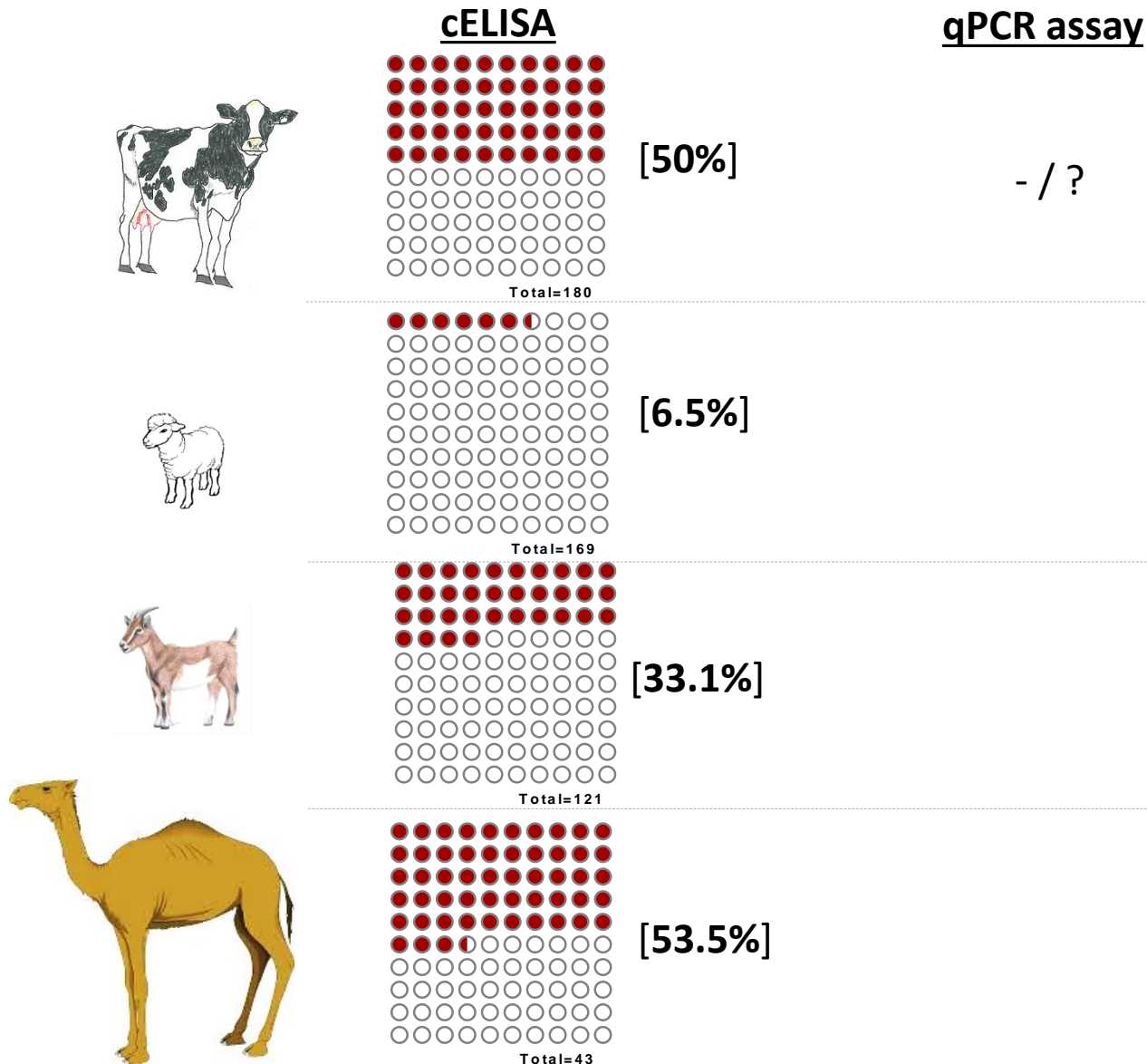
Total: 513



1
herd



BTV in Machakos - Results



Validating the BTV qPCR Assay

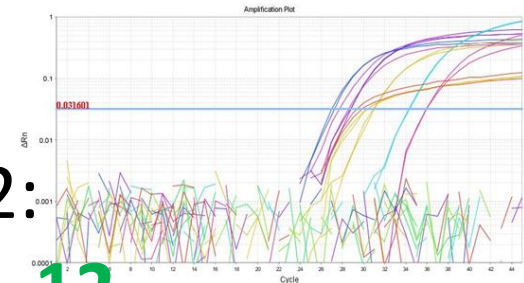
- BLUVAX vaccine [KEVEVAPI]
 - (BTV 1, 2, 3, 4, 8, 12)
- Panel of BTV-positive samples from Pirbright
 - (2018 BTV Ring Trial panel: BTV 1, 3, 4, 8)



BLUVAX™

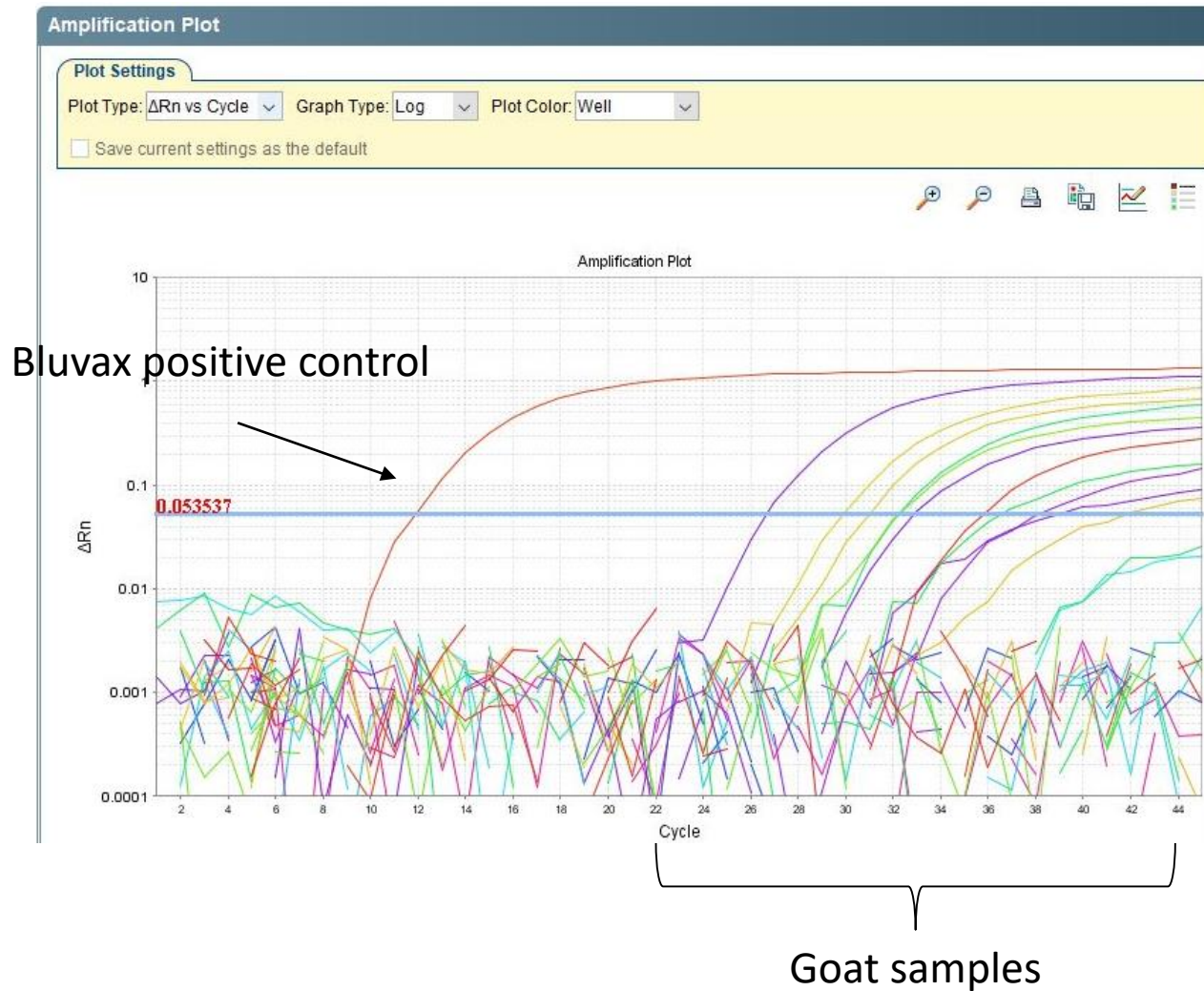
- qPCR with primers/probes for segment 10 (NS3): **functional**

- qPCR with primers/probes for VP2: **functional for serotypes 3, 4, 5, 8, 12**

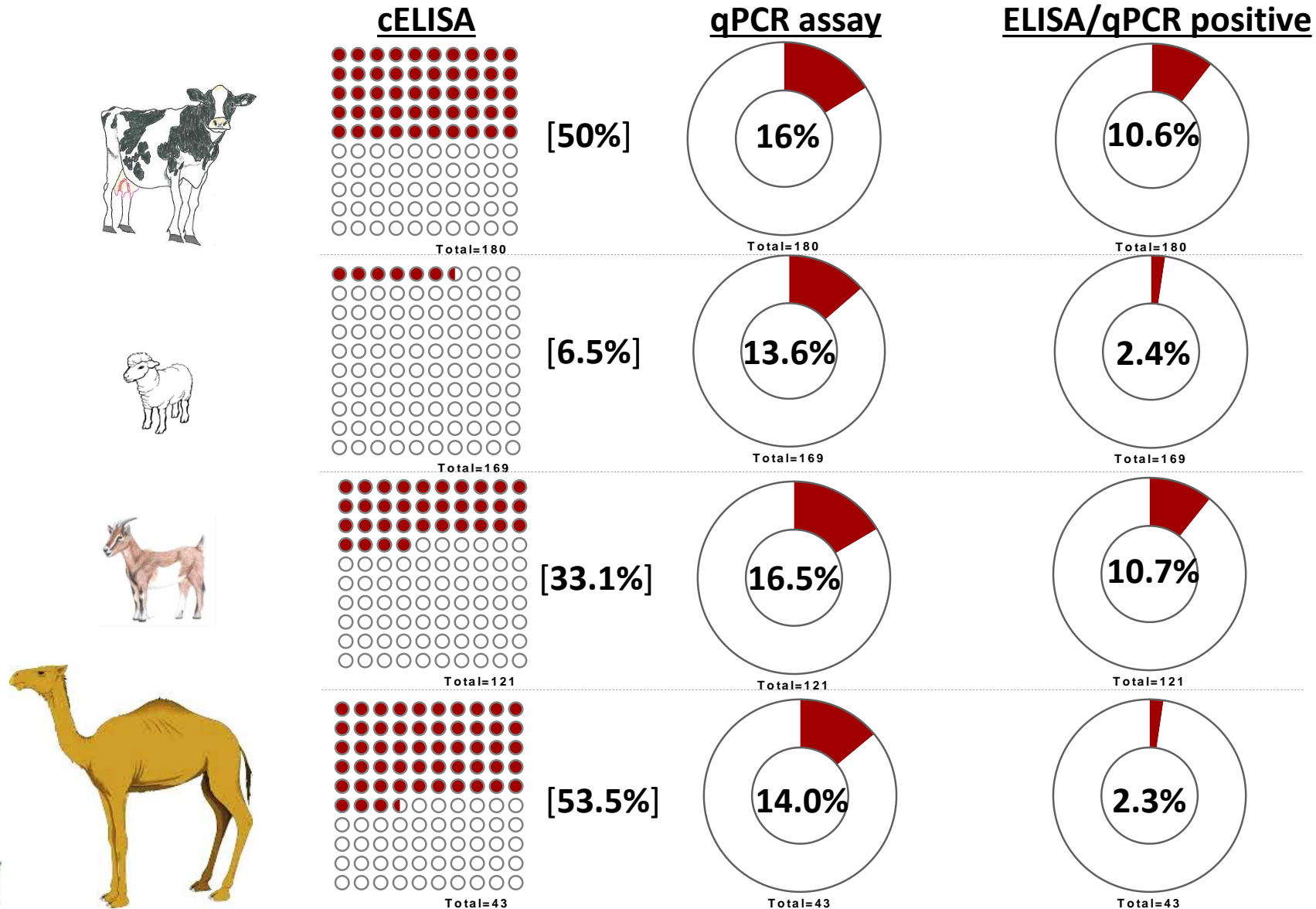


(RNA levels?, probe quality?, new serotype?)

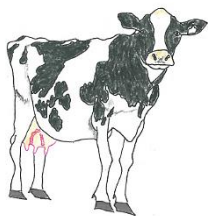
qPCR Assay with Goat Samples from Kapiti



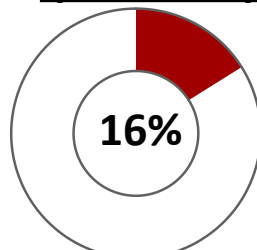
BTV in Machakos - Results



BTV in Machakos – Serotyping Results



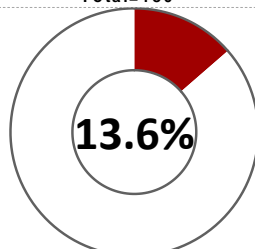
qPCR assay



Total=180

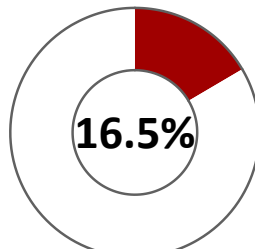
qPCR serotyping

BTV 4, 5, 8 [3]



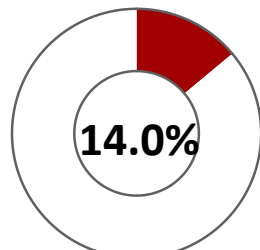
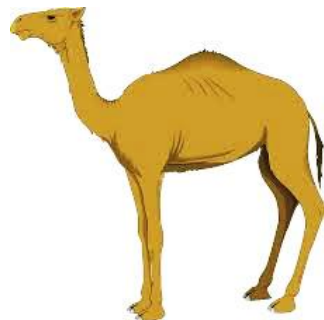
Total=169

BTV 8 [1]



Total=121

BTV 4, 8 [6]



Total=43

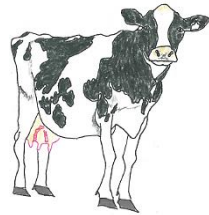
BTV ?

BTV in Isiolo - Results

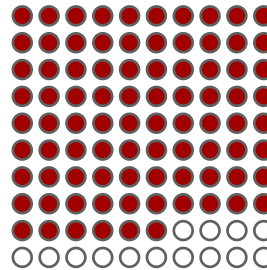


Isiolo: 285 km N of Nbi

Total: 259

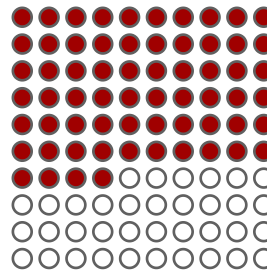
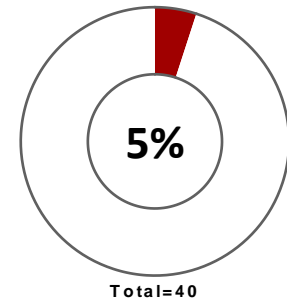


cELISA

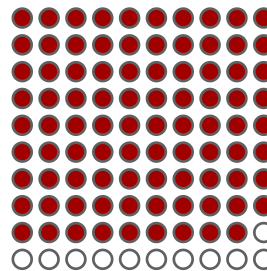
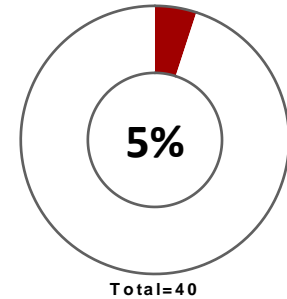


[86%]

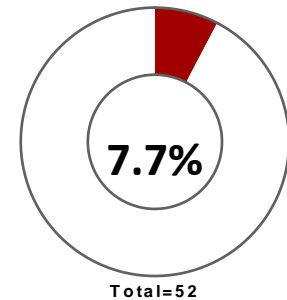
qPCR assay



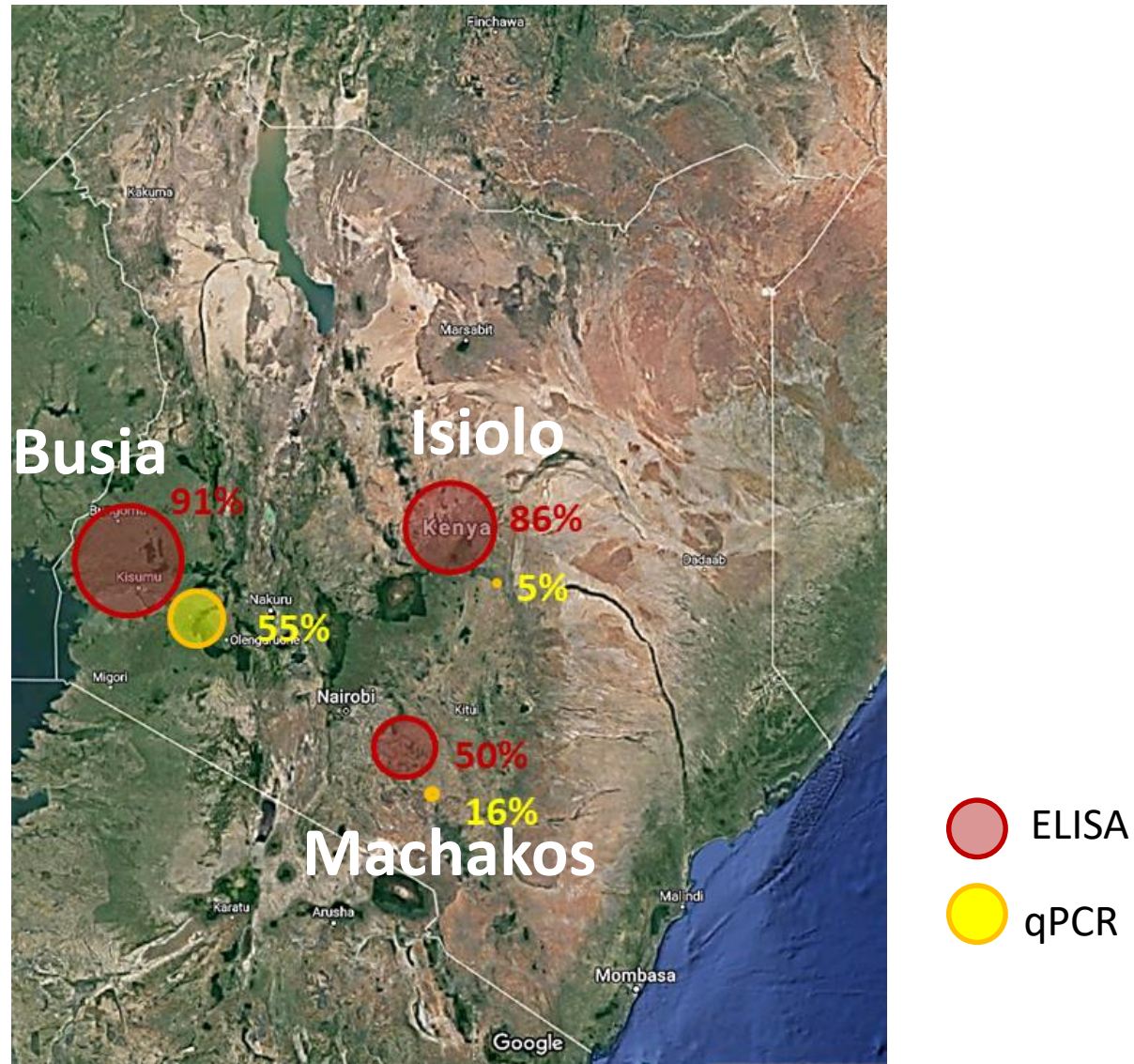
[64.1%]



[88.9%]



Exposure to BTV in Cattle from Different Regions of Kenya



Summary – ELISA / qPCR / Serotyping

- Higher prevalence in cattle and camel.
- Higher prevalence of BTV RNA in animals from Busia, followed by Machakos then Isiolo [very low]. (*Total of **972 samples** tested so far*).
- Kenyan sheep have been exposed to BTV [Machakos: 6.5%, Isiolo: 64.1%] without having caused apparent clinical infection [my assumption]?
- Able to type for BTV 3, 4, 5, 8, 12.

Hopefully, ^{not} We're caught in a trap
with BTV...

Karacké La vie en rose - Edith Piaf *

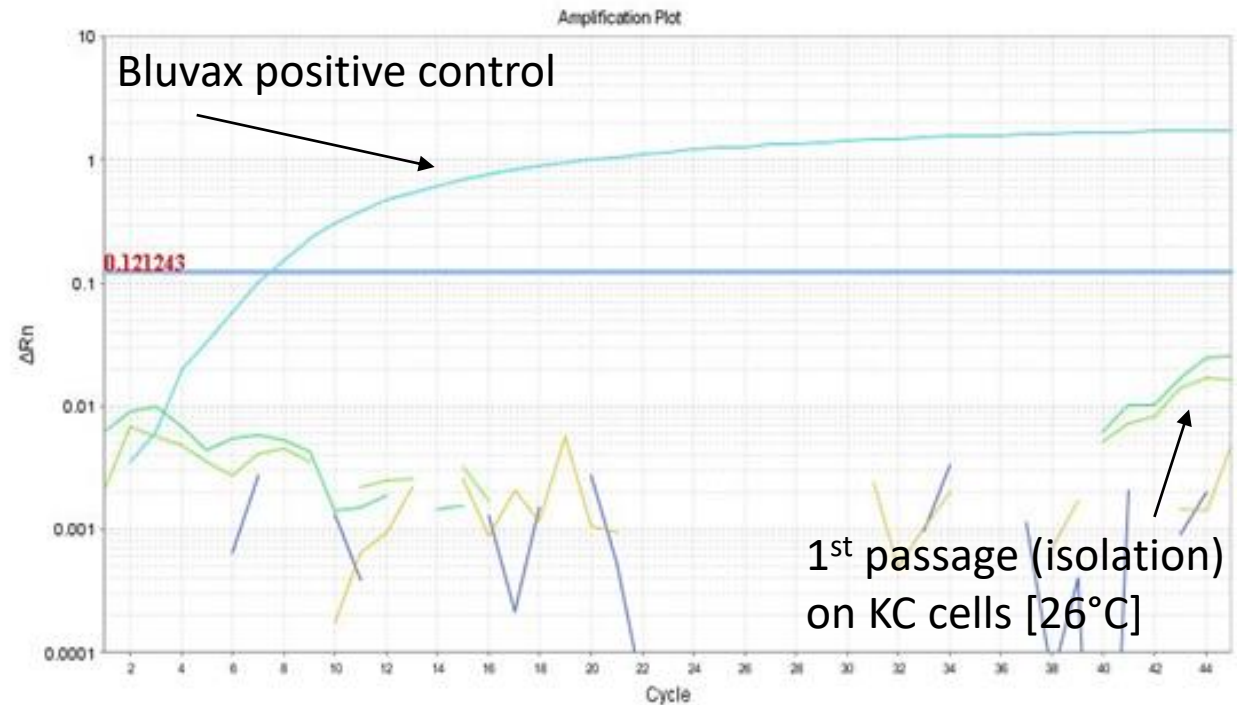
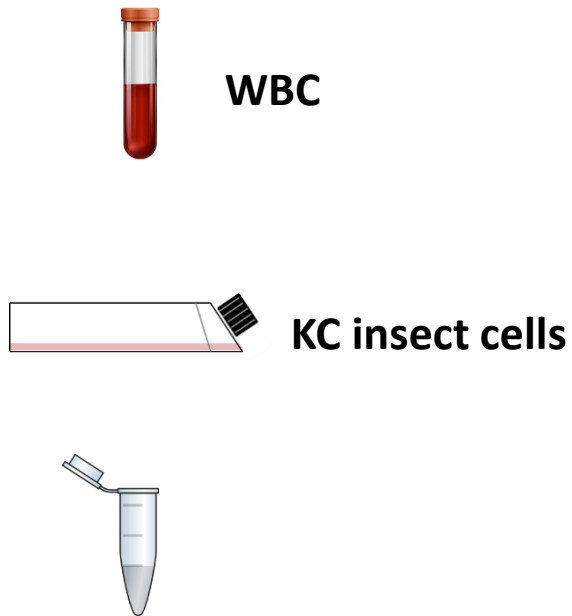
Voir la vie en rose...

Karafun.

Virus isolation from field samples
on KC and BHK cells

BTV Isolates from Kenya – First Trial

2 BTV isolates from sheep

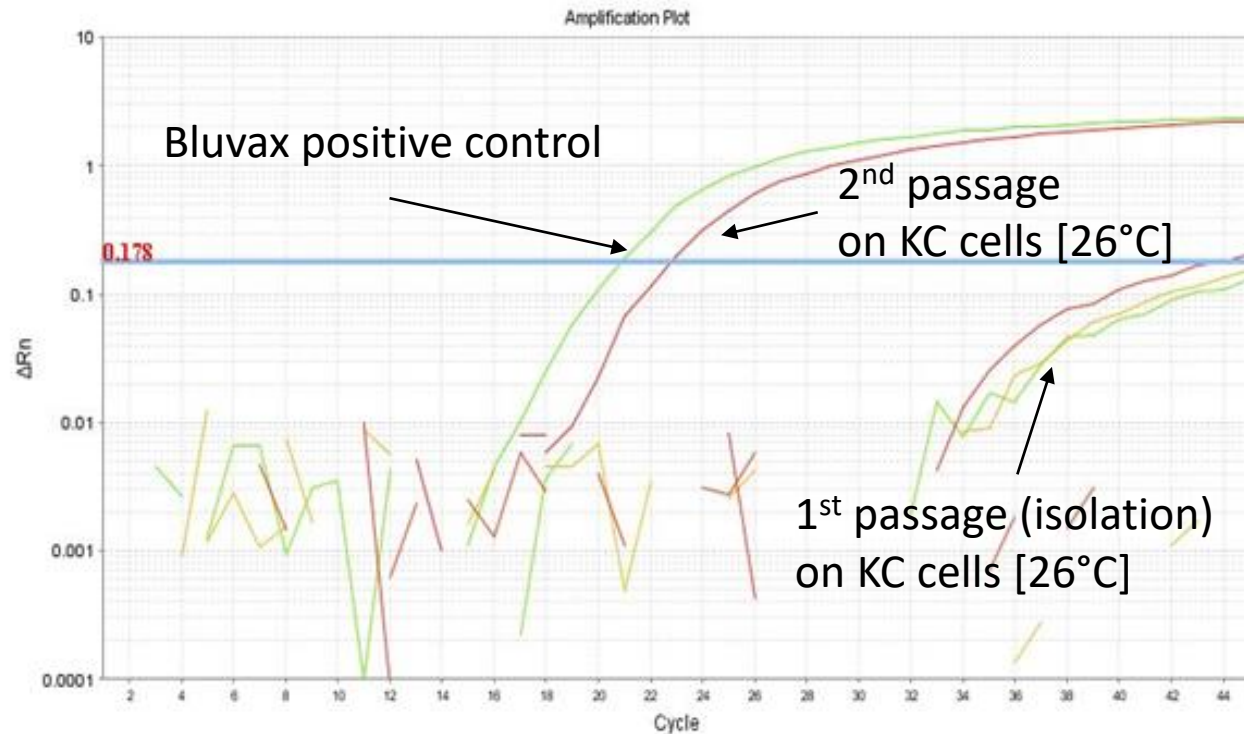
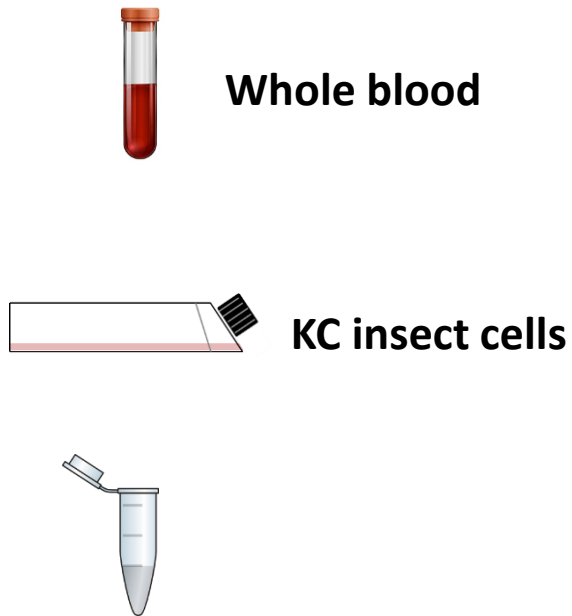


Initial Ct values from blood samples: 28.4, 30.4, 27.5

[Pirbright's cutoff value for virus isolation: Ct of 27 or lower]

BTV Isolates from Kenya – Second Trial

3 BTV isolates from sheep (BTV4, 8, ?)

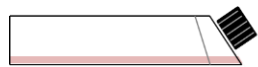
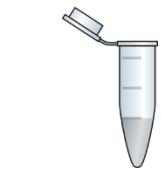


Initial Ct values from blood samples: 28.4, 30.4, 27.5

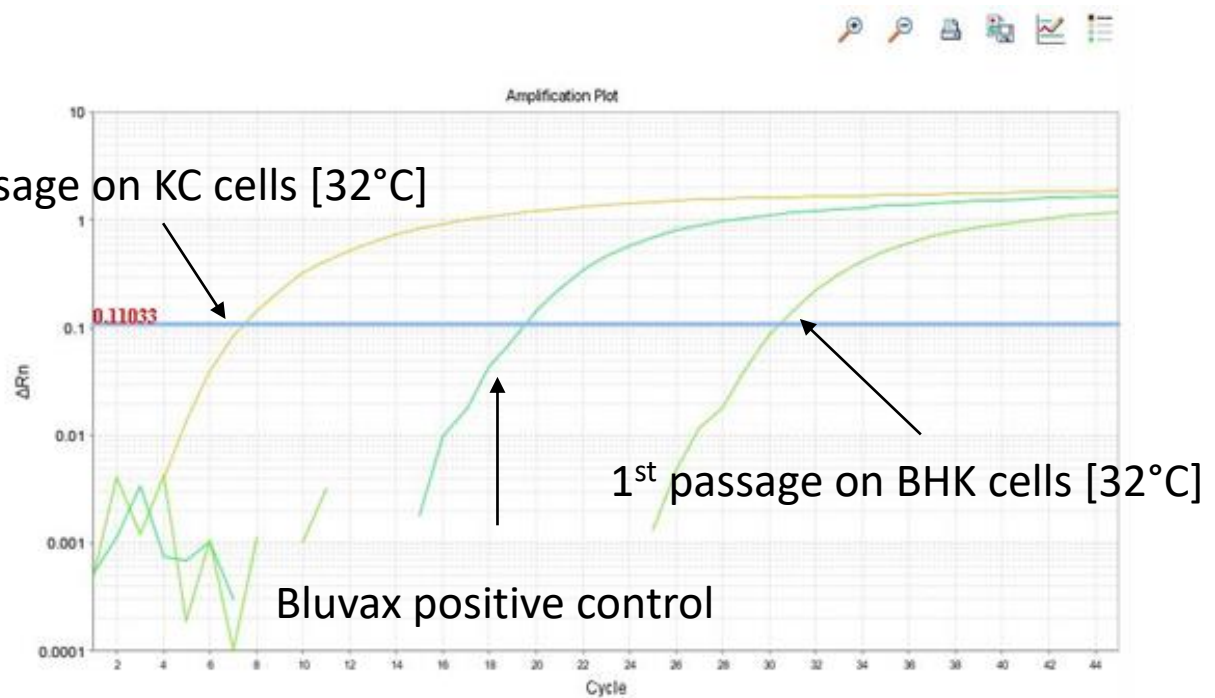
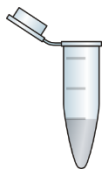
[Pirbright's cutoff value for virus isolation: Ct of 27 or lower]

BTV Isolates from Kenya – Trial on BHK Cells

1 BTV isolate from sheep

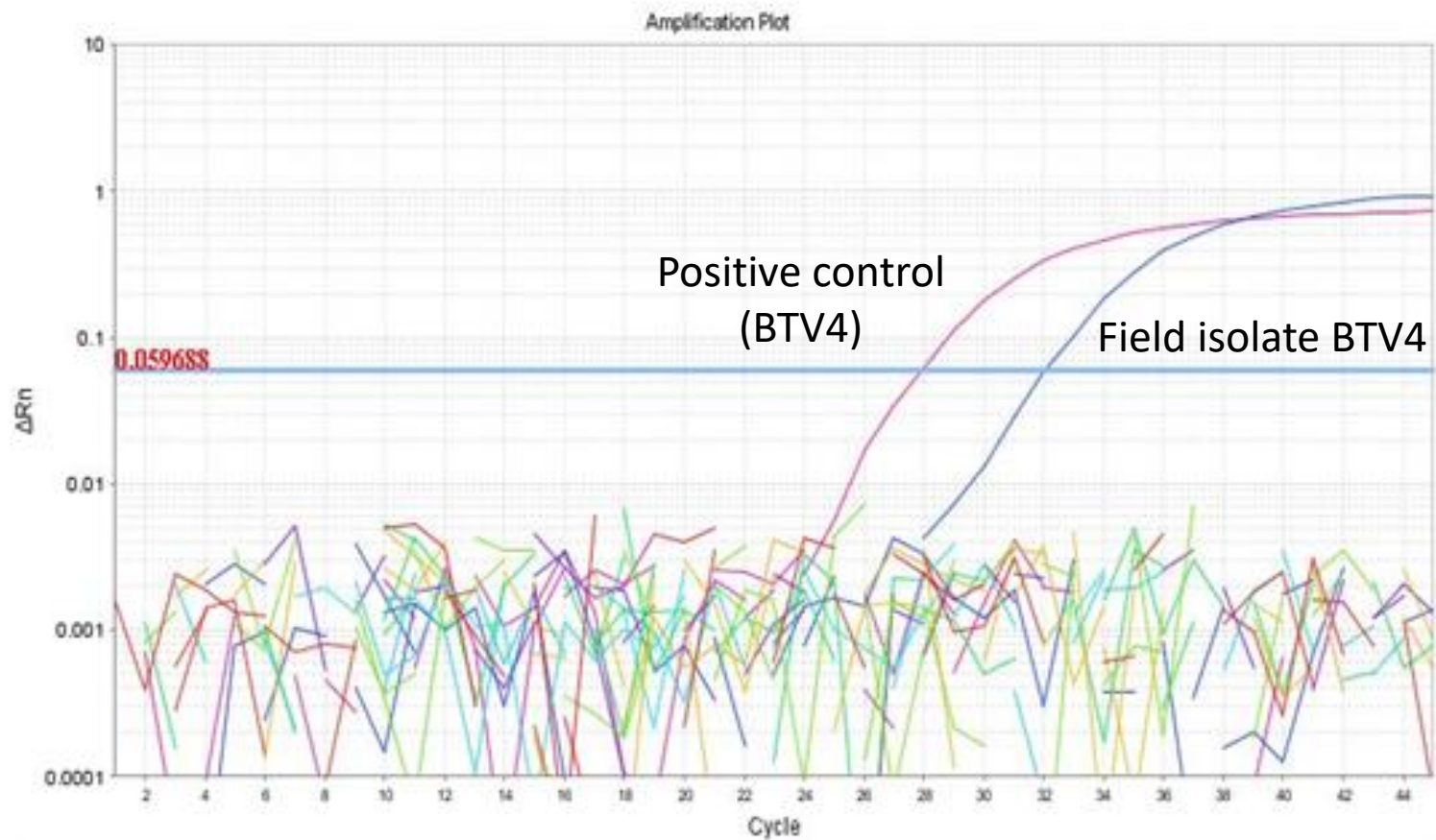


BHK cells



Serotyping of a field isolate (sheep) – BTV4

Screening with BTV-1 to BTV-26 serotype specific probes
[we are only sure that probes for BTV-3,4,5,8,12 work]



Summary – Bluetongue Virus Isolation

- 3 field isolates from sheep.
- 1 BTV 4, 1 BTV 8, one unknown.
- Able to expand virus from one isolate on KC and BHK cells.
- Able to isolate virus from whole WBC as well as from whole blood.

Planning for Next Year & Questions to be Answered

- Obtain samples from Mali & Uganda [ILRI ongoing projects].
- Plan a sampling expedition in Sudan or a country in West Africa [Benin?, Nigeria?].
- Continue qPCR typing and get more field isolates.
- Protocols for plaque-purification of virus isolates?
- Looking at the “BTV RNA conversion rate” instead of Ab conversion rate throughout the year?
- Why are Kenyan sheep more resistant to BTV?

Acknowledgments

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Josiah Njeru



Dr Carrie Batten
Dr John Flannery

NV Reference Lab



RESEARCH
PROGRAM ON
Livestock



European Commission



PALE-Blu


Understanding pathogen, livestock, environment
interactions involving bluetongue





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