CBPP Control Tools:

The Potential Contribution of Reference Laboratories

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Second meeting of SGE on CBPP of the GF-TADs for AfricaLusaka, Zambia, 23-25 July 2024

AU-PANVAC Laboratories

OUTLINE

- Current CBPP Vaccines Strains
- Production capacity of CBPP Vaccines in Africa
- WOAH guidelines for production CBPP Vaccine and Genetic Drift of CBPP Commercial Vaccines
- Antibio-therapy of CBPP and the challenges
- CBPP Diagnostics and Development of new Tools
- Conclusion

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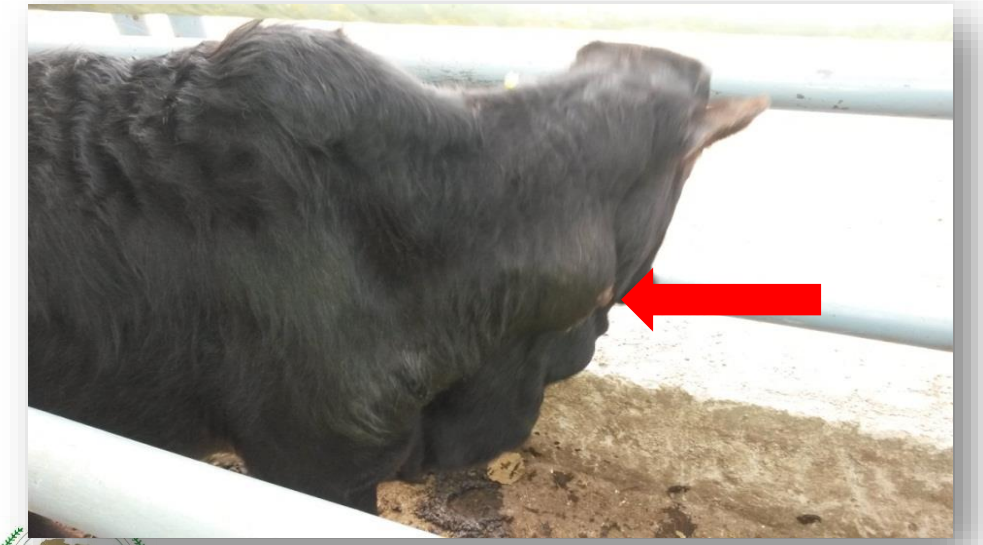
CBPP Vaccine strains: T1/44 Strain

- From a naturally mild strain isolated in **1951 in Tanzania** ***(Sheriff & Piercy, 1952)*** and followed by **44 passages** on eggs.
- Duration of Protection: **At least 12 Months (Annual vaccination)**
- Residual virulence, with occasionally **adverse post-vaccinal reactions** *"Willems reaction"*



- Local inflammatory reactions – *on injection site*
- Unpredictable frequency - *breed-dependent*?
- Post-vaccination reactions can generate resistance from farmers

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CBPP Vaccine strains: T1sr Strain

- Derived from T1/44 after 4 passages in mediun containing streptomycin (*A. Provost, Bull epizoot Dis Afr, 17, 1969*)
- **Streptomycin-resistant**
- **Don't induce Post-Vaccinal Reactions** at the injection site
- Used when combined campaigns against rinderpest and CBPP
- Duration of Protection: Shorter, estimated at **6 months (Bi-annual vaccination)**

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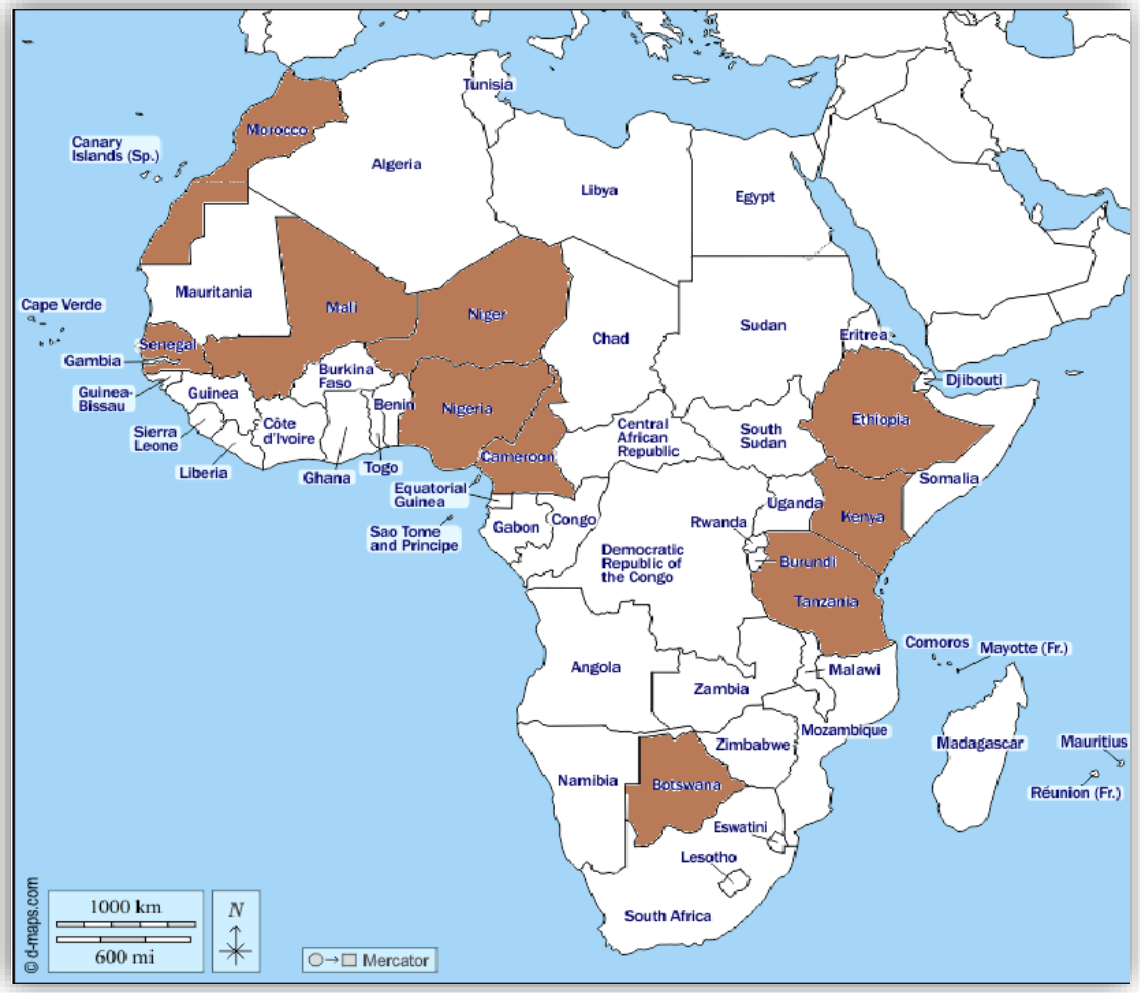
Production Capacity of CBPP vaccines

❖ African Manufacturers

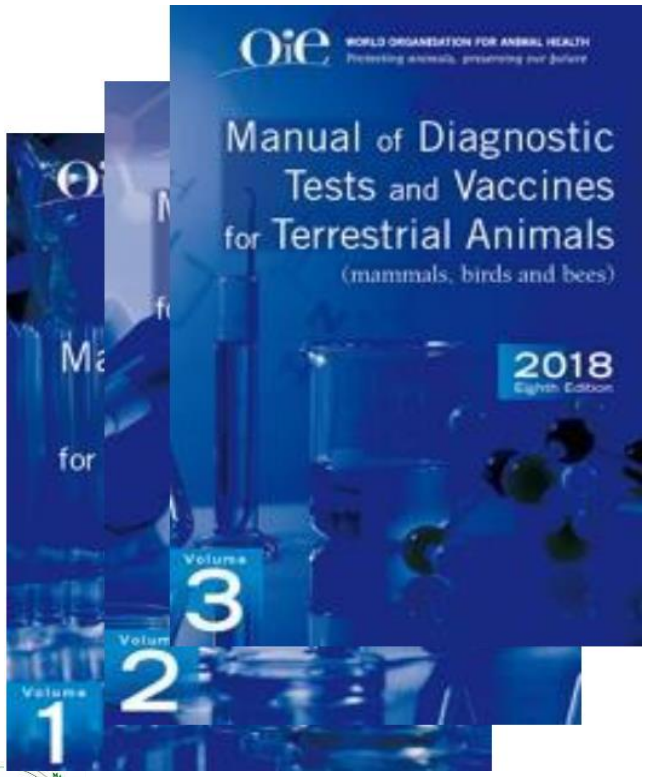
- Northern Africa: **MOROCCO** (MCI)
- Western & Central Africa
  - **CAMEROUN** (LANAVET)
  - **MALI** (LCV)
  - **NIGERIA** (NVRI)
  - **NIGER** (LABOCEL)
  - **SENEGAL** (ISRA-Production)
- Eastern Africa
  - **ETHIOPIA** (NVI)
  - **KENYA** (KEVEVAPI)
  - **TANZANIA** (TVI, HESTER)
- Southern Africa: **BOTSWANA** (BVI)



❖: Oversea CBPP Manufacturer: JOVAC (Jordan)

Vaccine Quality Control Tests

Five Major Vaccine QC Tests conducted based on the *"Manual of Diagnostic Tests and Vaccines for Terrestrial Animals"* of the World Organisation for Animal Health Founded as OIE (WOAH)



- **Identity**
- **Purity/Sterility**
- **Safety/Innocuity**
- **Potency/Efficacy**
- **Stability**

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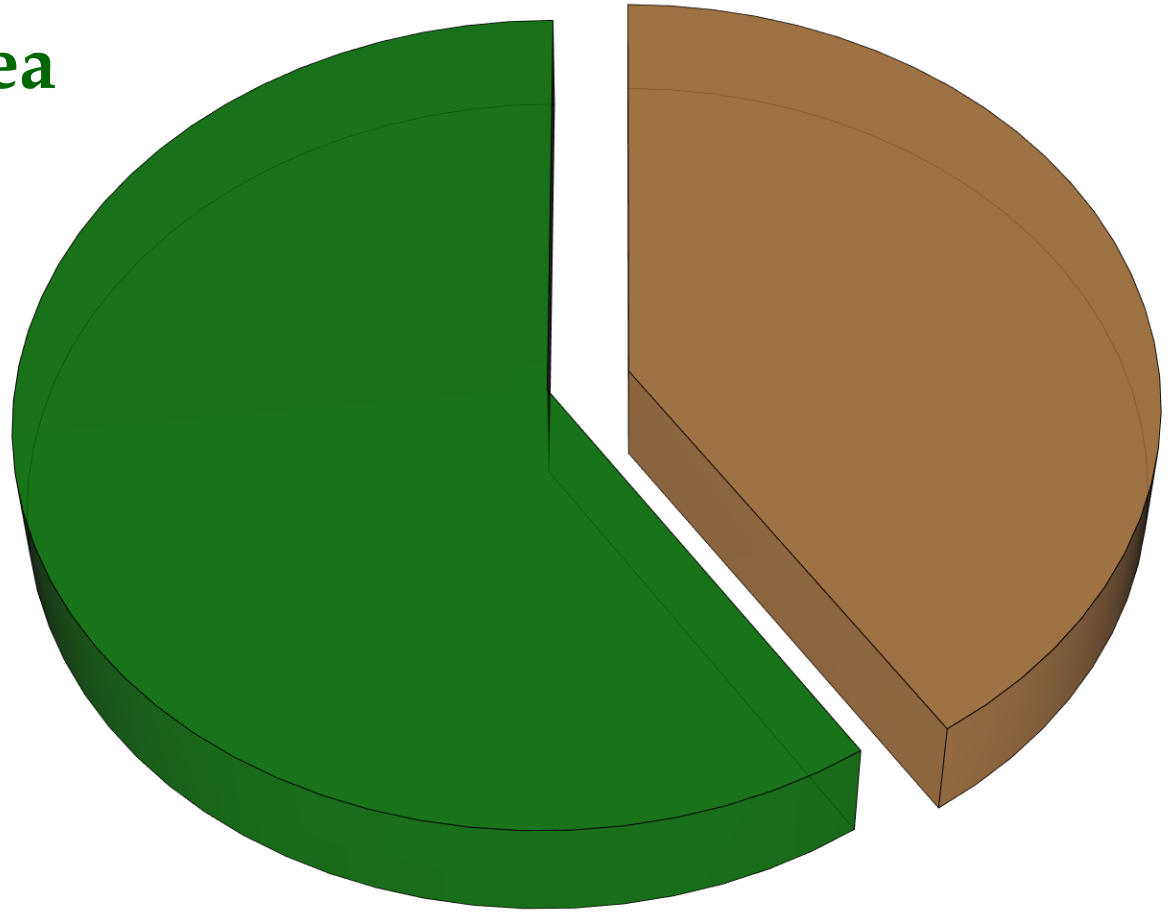
Certified CBPP Vaccine Doses: African & Oversea Manufacturers 2018-2022

Agend 2063
The Africa we Want

Oversea
59%



African Manufacturers
41%

Require the need to increase CBPP Vaccine production capacity in Africa

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Certified CBPP T1/44 & T1sr Vaccines

Vaccine Strain	2018	2019	2020	2021	2022
T1/44 (Batches)	28 (82.4%)	38 (84.4%)	24 (92.3%)	6 (55.5%)	25 (86.2%)
T1/44 (Doses)	37,781,400 **(77%)**	112,129,650 **(82.4%)**	60,182,050 **(97.3%)**	45,420,000 **(85.2%)**	39,440,000 **(83.2%)**
T1sr (Batches)	6 (17.6%)	7 (15.6%)	2 (7.7%)	5 (45.5%)	4 (13.8%)
T1sr (Doses)	11,352,900 **(23%)**	23,973,550 **(17.6%)**	1,639,350 **(2.7%)**	7,904,300 **(14.8%)**	7,985,100 **(16.8%)**
Total (Doses)	**49,134,300**	**136,103,200 ***	**61,821,400**	**53,324,300**	**47,425,100**

*** PRAPS Project**

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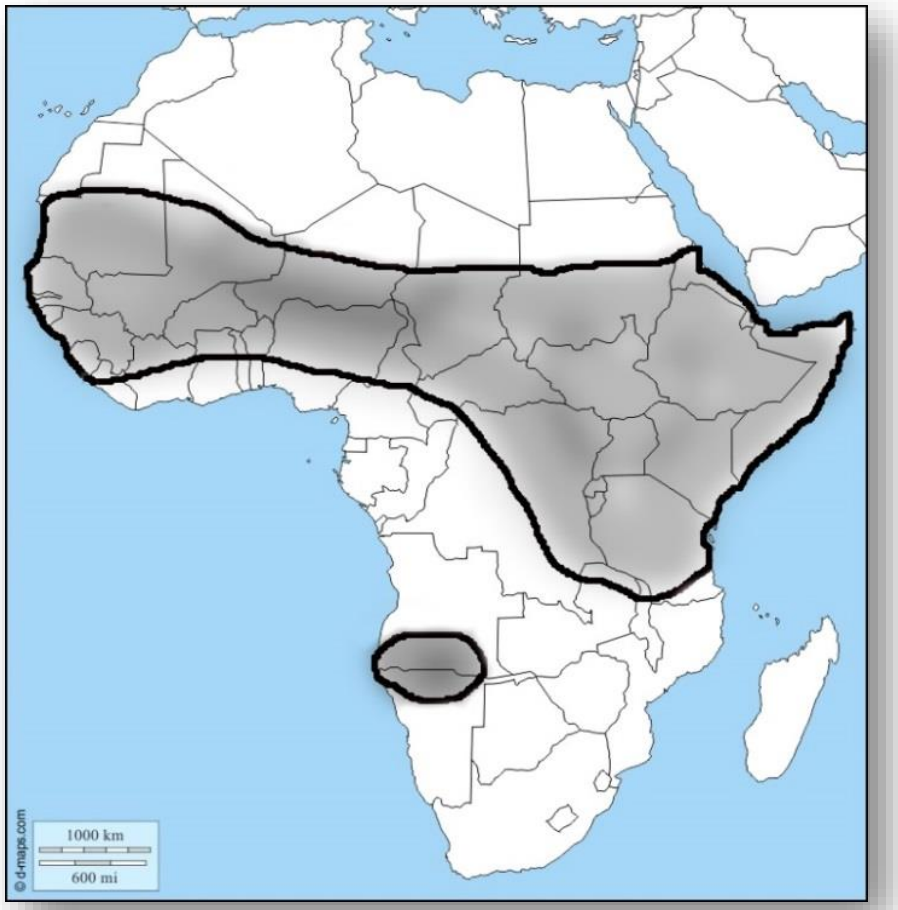
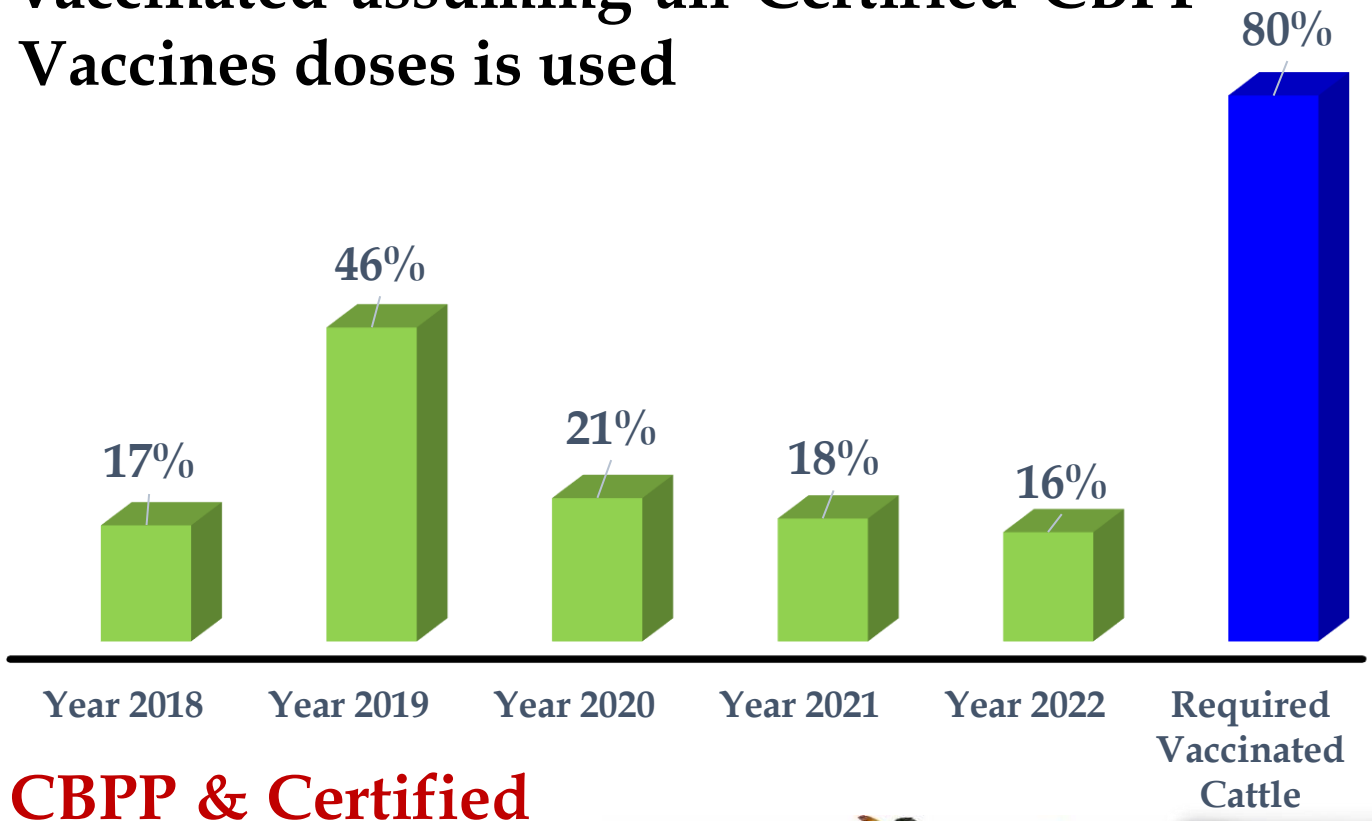
Cattle Population & Certified CBPP Vaccines

❖ Cattle Population in Africa at risk of CBPP infection: 370,000,000 heads



❖ Maximum of Cattle Population vaccinated assuming all Certified CBPP Vaccines doses is used



❖ Gap in Production of CBPP & Certified vaccines for protection of cattle at risk

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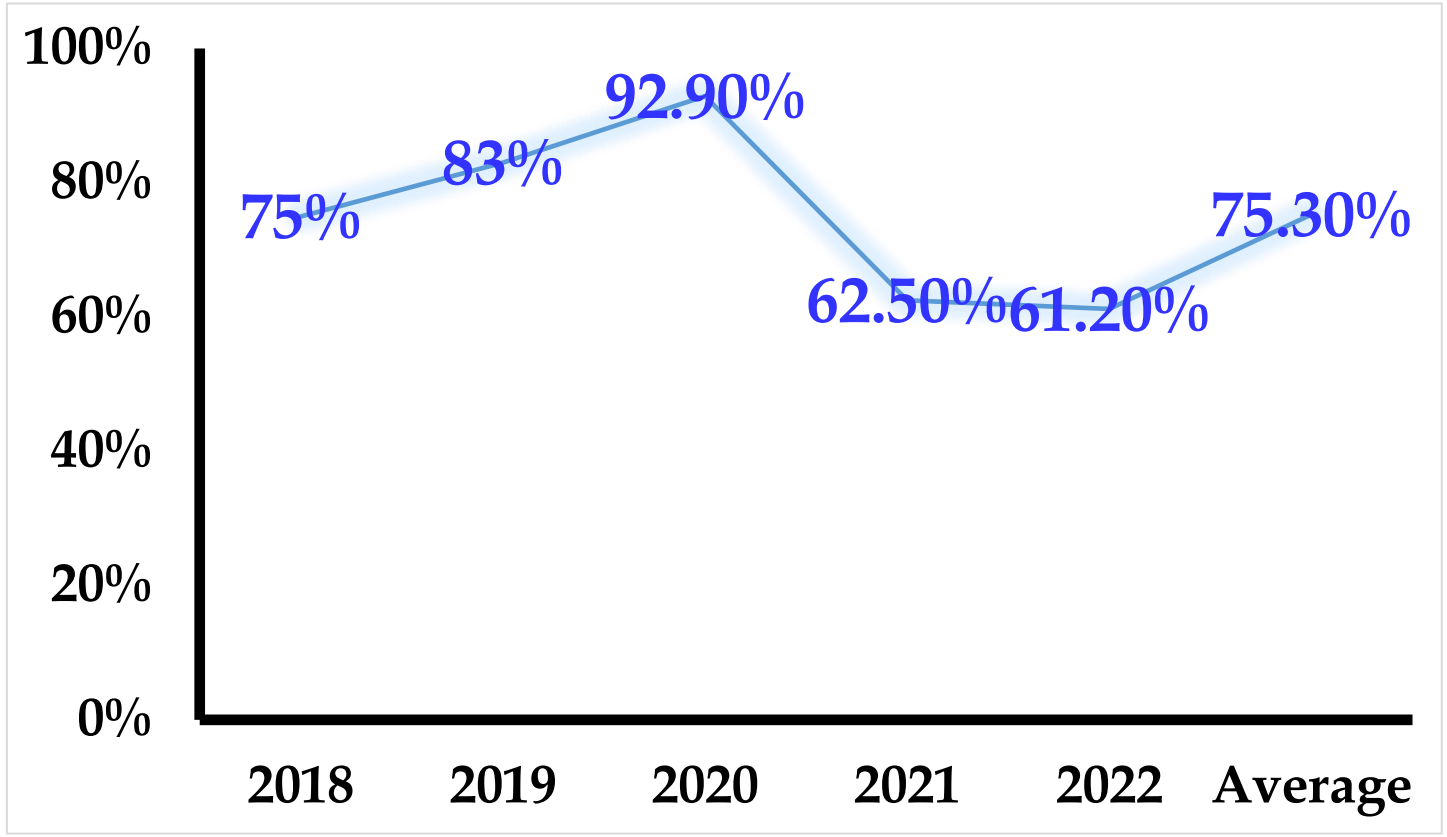
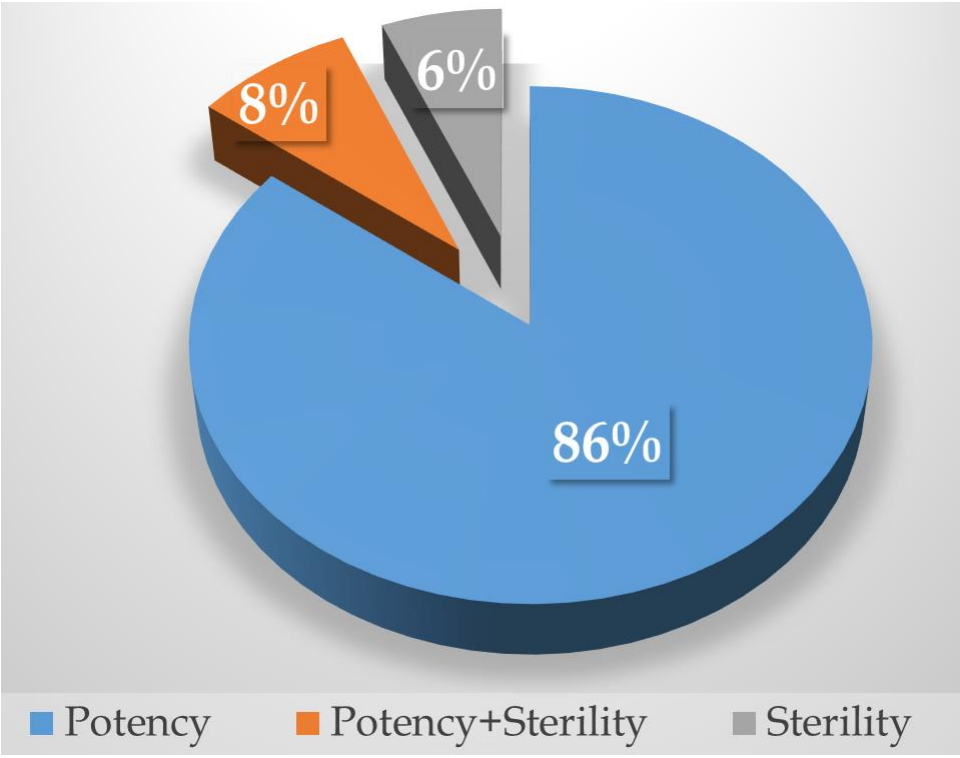
CBPP Vaccine Quality: Certified Pass Rate





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Guidelines for Production CBPP Vaccine

Terrestrial Manual: Chapter 3.4.8. – Contagious bovine pleuropneumonia (infection with Mycoplasma mycoides subsp. mycoides)

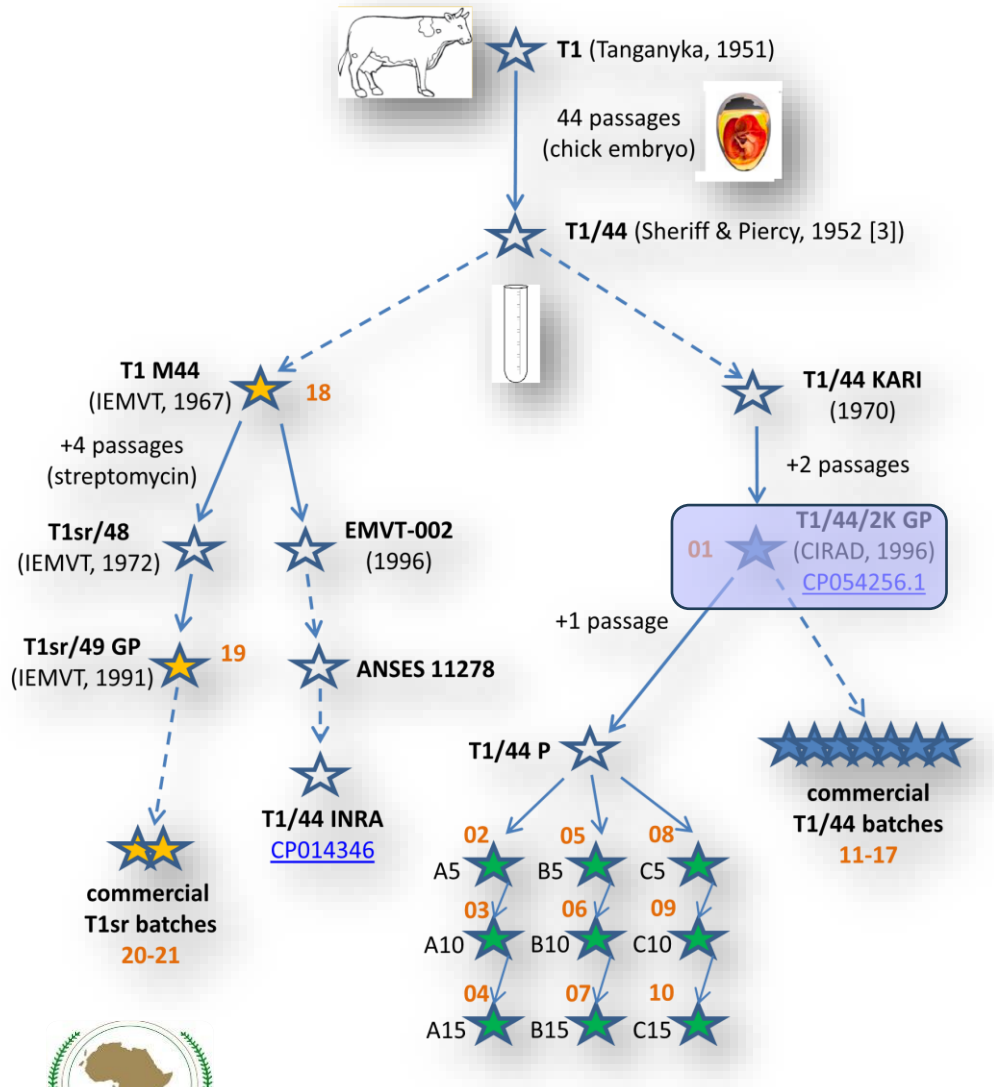
- Limited number of **passages** between the parental strain stock and the final vaccine batch

  *"Vaccine bulk cultures must be obtained with a maximum of **3 successive passages** of the master seed"*

- **Cloning procedures must be avoided**.

To avoid selection of variants which could impact on the Protection

Genetic Drift of CBPP Commercial Vaccines



Contents lists available at ScienceDirect

Vaccine 42 (2024) 1868–1872

journal homepage: www.elsevier.com/locate/vaccine

Deep sequencing and variant frequency analysis for the quality control of a live bacterial vaccine against contagious bovine pleuropneumonia, strain T1

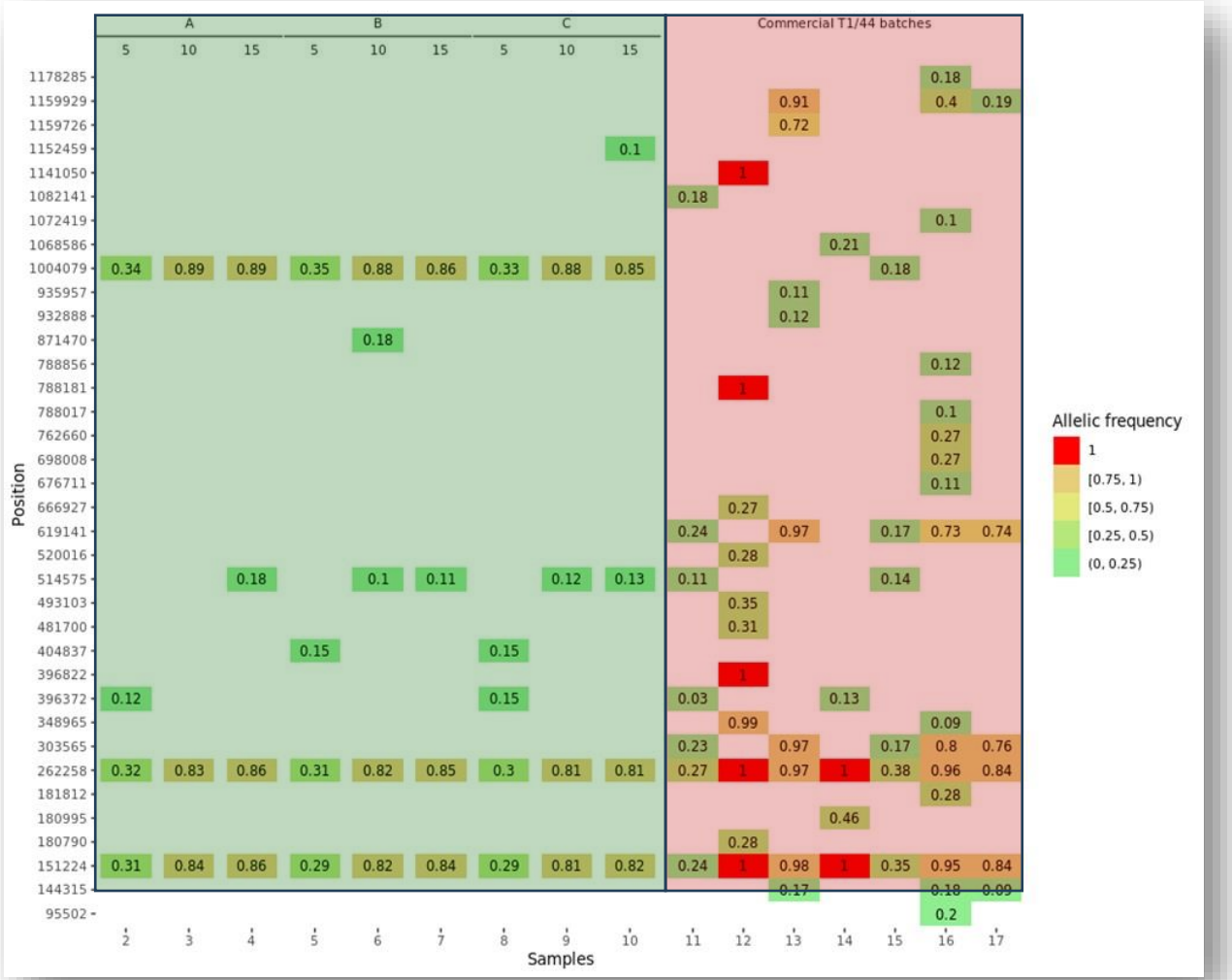
François Thiaucourt [a,b], Antoni Exbrayat [a,b], Etienne Loire [a,b], Anne Boissi`ere [a,b], Nick Nwankpa [c,1], Lucía Manso-Silvan´ [a,b,*]

- *CIRAD, UMR ASTRE, F-34398 Montpellier, France*
- *ASTRE, Univ Montpellier, CIRAD, INRAE, F-34398 Montpellier, France* [c]
- *Pan-African Veterinary Vaccine Centre of the African Union (AU -PANVAC), PO Box 1746, Bishoftu, Ethiopia*

- 3 experimental cultures A, B & C were performed with passaged 15 times
- 7 commercial T1/44 vaccine batches
- 2 commercial T1sr vaccine batches

Genetic Drift of CBPP T1/44 Commercial Vaccines

❖ Single Nucleotide Polymorphism (SNP) positions identified in each sample compared to the T1/44/2KGP genome

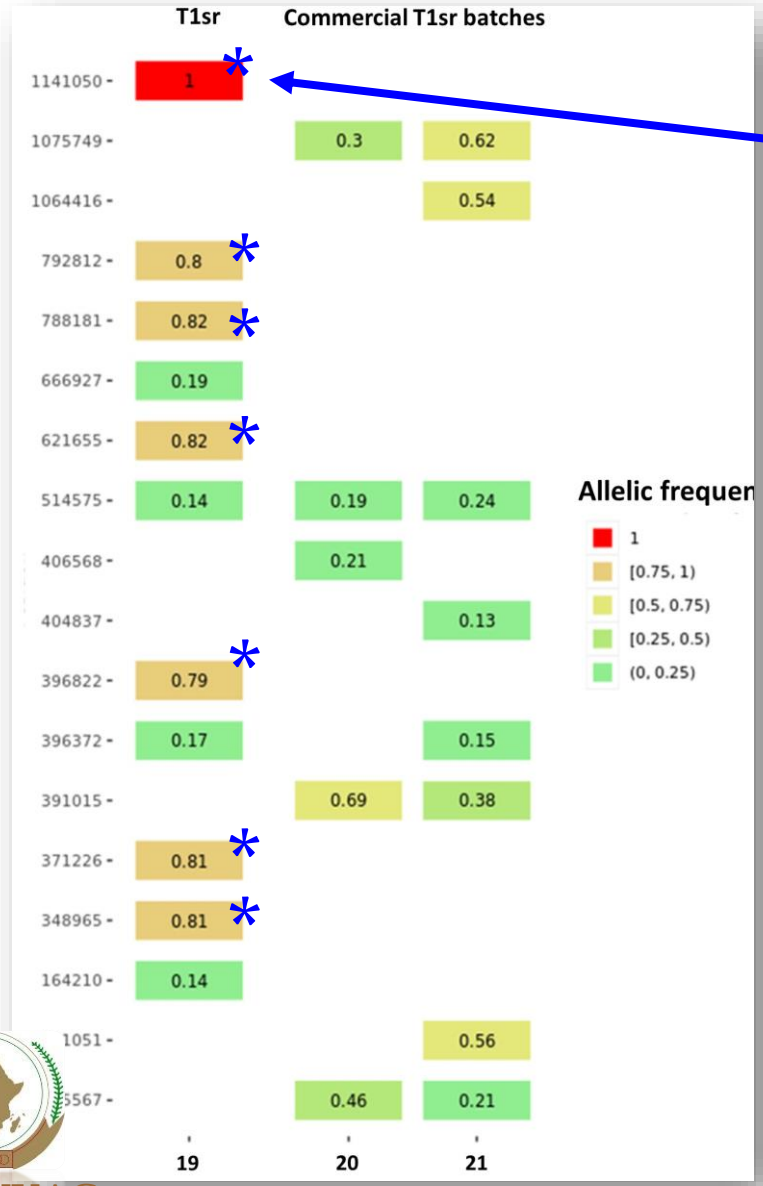


❖ Experimental cultures A, B & C cumulated **few mutations with identical SNP**, reaching ≤ 89% after 15 passages, and a few other at low frequency.

❖ Commercial T1/44 vaccine batches showed **Contrasted Results**:

- 11 & 15 were similar to the GP stock, indicating following GMP;
- 16 presented higher number and frequency of **mutations indicating additional subcultures**;
- 13 & 17 had SNP at high frequency, and
- 12 & 14 showed several fixed SNPs, reflecting the selection of variants due to cloning

Genetic Drift of CBPP T1sr Commercial Vaccines



Fixed, non-synonymous mutation in the *rplS*gene encoding 30S ribosomal protein S12, correlated with **streptomycin resistance**, but also reduced growth and virulence (4).

- The T1sr GP stock presented **7 characteristic SNPs** * at high frequency, including an *rplS* mutation.
- The commercial batches tested had lost some of these SNPs.
- Manufacturers may have removed the streptomycin pressure during the growth

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Improving Production of CBPP Vaccines

AU-PANVAC Project: Review CBPP Vaccine Production Process

01

- Determine optimal harvesting time for Mmm culture

02

- Compare cultivation methods: Shaking vs. Static

03

- Compare stabilizers for the freeze-drying process that maintain stable vaccine titer.

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CBPP New Vaccines Developments

- *Mmm* bacterins in oil emulsion or formulated with Freund's Complete Adjuvant induced good protection and improved thermostability (*Garba et al. 1986; Mwirigi et al., 2016*).
- A negatively marked inactivated *Mmm* strain in oil adjuvant provided very promising results (*VACNADA project, CIRAD unpublished data*): DIVA approach
  - Proof of concept for a protective immune response
  - Proof of concept to monitor vaccinated cattle through cELISA CBPP.
  - ***NEED A VALIDATION PROCESS***
- Bivalent live attenuated vaccine formulation combining T1/44 and LSD virus showed to be safe and immunogenic *(Safini et al., 2022)*: **Target CBPP & LSD control**
- Effort on development of subunit, recombinant vaccines with very limited success *(Abusugra et al, 2000, Huebschle et al, 2003, Mulongo et al, 2013).*

CBPP Antibio-Therapy and the Challenges

- The control of CBPP is mainly based on vaccination, since antibiotic treatments are discouraged due to the risk of residues in meat and milk and AMR.

- **Antibiotic treatments**

  - Have to be carried out under the direct control of **Veterinarians or Animal Health Technicians**. (Which is feasible in most African regions)

  - Can reduce morbidity, mortality, and production losses caused by CBPP infection.

  - Showed to have a considerable impact on the transmission of CBPP and reduction of number of infecting animals.

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CBPP Diagnostics Tools: Mmm detection by PCR

Conventional PCR

- End-point detection
- Need post-PCR analysis (electrophoresis)
- Risk of contamination (PCR products)
- Not quantitative; only qualitative results
- Limited sensibility

-PCR + REA (Bashiruddin *et al*, 1994)

-Nested PCR (Miserez *et al*, 1997): **Increased risk of contamination**

-Mmm-specific PCR (Dedieu *et al*, 1994)

-Mmm T1-specific PCR (Lorenzon *et al*, 2000): QC of T1 vaccines at PANVAC

Real Time PCR

- Early detection
- Direct detection
- Lower contamination risk
- Quantitative (when optimised)
- Higher sensitivity > **Quality assurance**

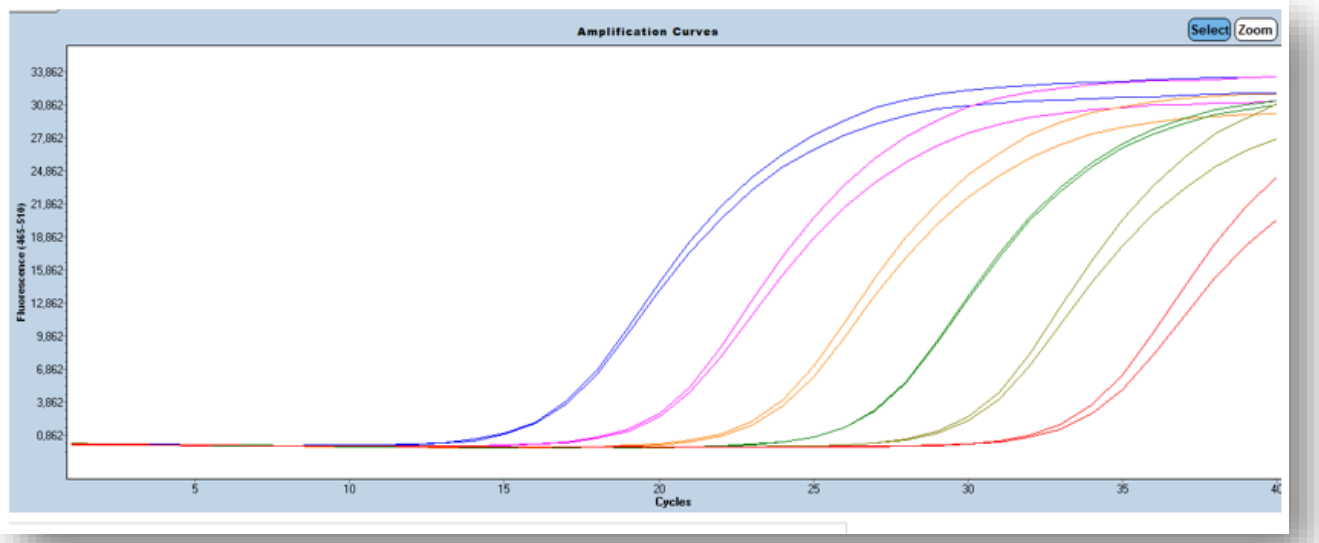
-SYBR Green-based (Fitzmaurice *et al*., 2008; Lorenzon *et al*, 2008)
-TaqMan-based (Gorton *et al*, 2005; Schnee *et al*, 2011)

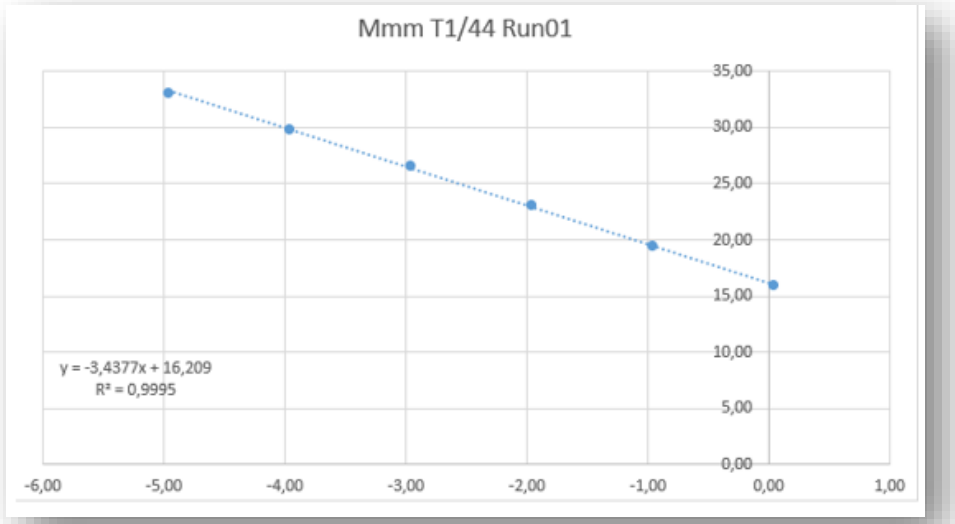
Commercial kits (Creative Biogene, NY, USA; Ring Biotechnology, Beijing, China; MyBioSource, Inc., San Diego, USA).
No validation / Not for diagnostic purposes (for research use only)!!!

CBPP Diagnostics Tools: Mmm detection by PCR

❖ **New RT PCR development (CIRAD):**

Specific TaqMan-based *RT PCR, following DNA extraction directly from suspected samples (under validation for ISO 17025 accreditation)*





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CBPP Diagnostics Tools: CBPP Serology Tests

❖ Complement Fixation Test (CFT)

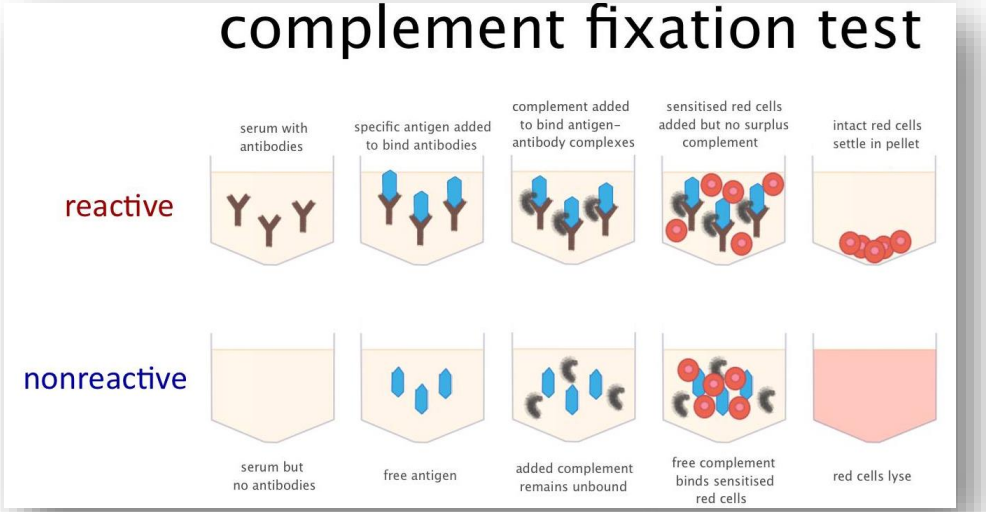
Advantages:

- **Early detection (IgM)**
- **Relatively cheep**
- Little equipment required
- **For diagnosis at herd level**, eradication programmes and import clearance

Disadvantages:

- Sensitivity rapidly declines (IgM)
- Lack of specificity (~2% false positive results)
- Difficult to standardize
- Experienced personnel required

Commercial kits: BORA Tech Ltd Nairobi, Kenya; CIRAD Ref Lab, France



❖ Shortages and long delays to supply:

AU-PANVAC will support the production and distribution CBPP CFT reagents

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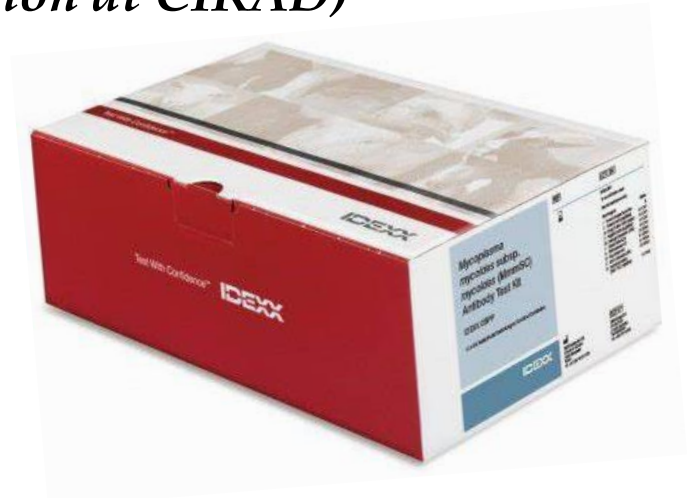
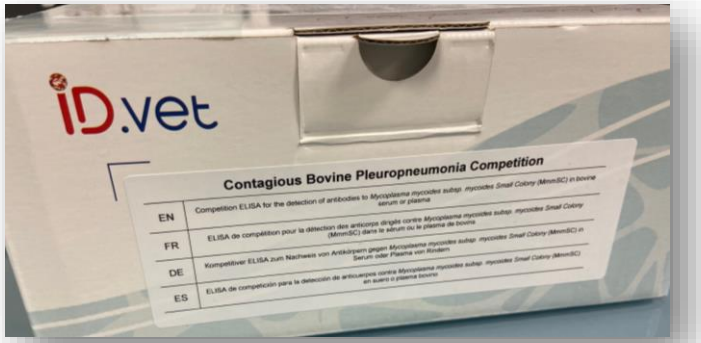
CBPP Diagnostics Tools: CBPP Serology Tests…

❖ **cELISA Test: Commercial kit (IDEXX > IDvet)**

IDEXX kit, discontinued!* > *Transferred to IDvet, now commercially available* (*under validation for ISO 17025 accreditation at CIRAD)





- Advantages:
  - Highly specific: >99.9% (Mab)
  - Longer detection (IgG): chronic stages
  - Suited for prevalence studies, eradication programmes and import clearance

- Disadvantages:
  - Later detection than CFT (IgG)
  - Equipment (plate reader) required
  - Experienced lab/personnel required (quality management)

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CBPP Diagnostics Tools: CBPP Serology Tests…

❖ AU-PANVAC cELISA Test Development

Veterinary Medicine International

Forward Series

Research Article
Open Access

Development and Evaluation of Monoclonal Antibodies against CBPP Antigen with the End Goal of Developing an ELISA Kit

Lorato Ramathudi-Dunbar, Emmanuel Awosanya, Sanne Bodjo Charles, Ethel Chitsungo, Cisse Rahamatou Moustapha Boukary, Nick Nwankpa, Hassen Gelaw … **See all authors**

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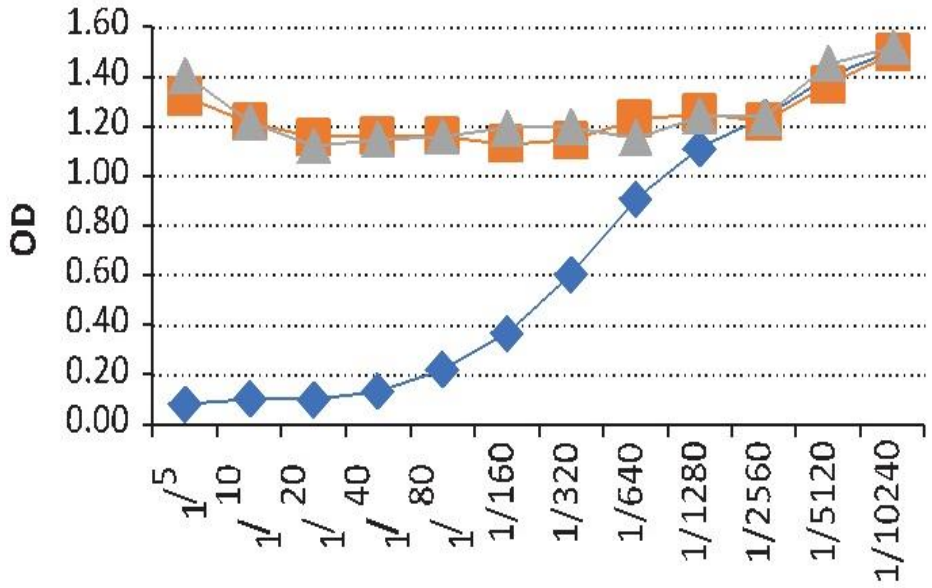

New CBPP cELISA assay under validation at AU-PANVAC

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CBPP Diagnostics Tools: CBPP Pen side-Test

- **Slide agglutination test (SAT):** using colored antigen, which yields agglutination in the presence of positive sera containing IgMs.
- **Latex agglutination test (LAT):** using beads coated with Mmm polysaccharides:

  - Advantages:
    - Simple and fast
    - No lab requirements
    - Early detection (IgM)
    - Suitable for early detection in the field
  - Disadvantages:
    - Lack of specificity (frequent cross reactions)

  Commercial kit "BoviLAT" (UK): ***Ceased (was too expensive)***

- **Lateral Flow Test (LFT):** for rapid serological screening (Heller et al., 2016)

  No industrial partner willing to produce at large scale and validate this test (limited market size implies small batches and high production costs).

No commercial pen-side tests are currently available for rapid CBPP diagnosis in the field. Market research to assess affordable diagnostics.

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Nagoya Protocol

- An international agreement that **came into force in 2014**, governing access and benefit-sharing (ABS) with respect to genetic resources.
- It is relevant for a variety of **commercial and non-commercial sectors** involved in the **use and exchange of genetic resources**.
- The fundamental role of the agreement is to **prevent misappropriation of natural resources, through fair and equitable sharing of the benefits** arising out of the utilization of genetic resources is a universally supported concept.

- The Protocol **recognizes the rights of indigenous and local communities** to identify the rightful holders of their traditional knowledge **associated with genetic resources and to benefit from the use of these resources**.

Challenges in Implementation of Nagoya Protocol

1.Complexity of Implementation: Ensuring effective implementation of the protocol's provisions across different countries and regions can be challenging.

2.Compliance with Domestic Legislation: Parties need to take appropriate measures to ensure compliance with domestic laws and regulations related to access and benefit-sharing.

3.Dispute Resolution: Establishing mechanisms for resolving disputes related to mutually agreed terms, jurisdiction, and applicable laws can pose challenges.

4.Financial Resources: Adequate financial resources need to be considered for the implementation of the protocol, aligning with the provisions of the Convention.

5.Involvement of Indigenous and Local Communities: Ensuring the active involvement and capacity-building of indigenous and local communities, can be a challenge in practice.

CONCLUSION

- Study required to improve the production and quality of CBPP vaccine
- Combined vaccination and drug treatment programs can offer greater impact than either approach alone.
- In a primary outbreak of CBPP, under veterinary control, where it is not possible to apply stamping out, the use of antibiotics and subsequent vaccination, associated with the surrounding vaccination of neighbouring farms, could be a valid approach.
- Development of affordable diagnostics

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