

Diagnostic challenges with regards to CBPP and what the reference laboratories can do to help

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# Presentation outline

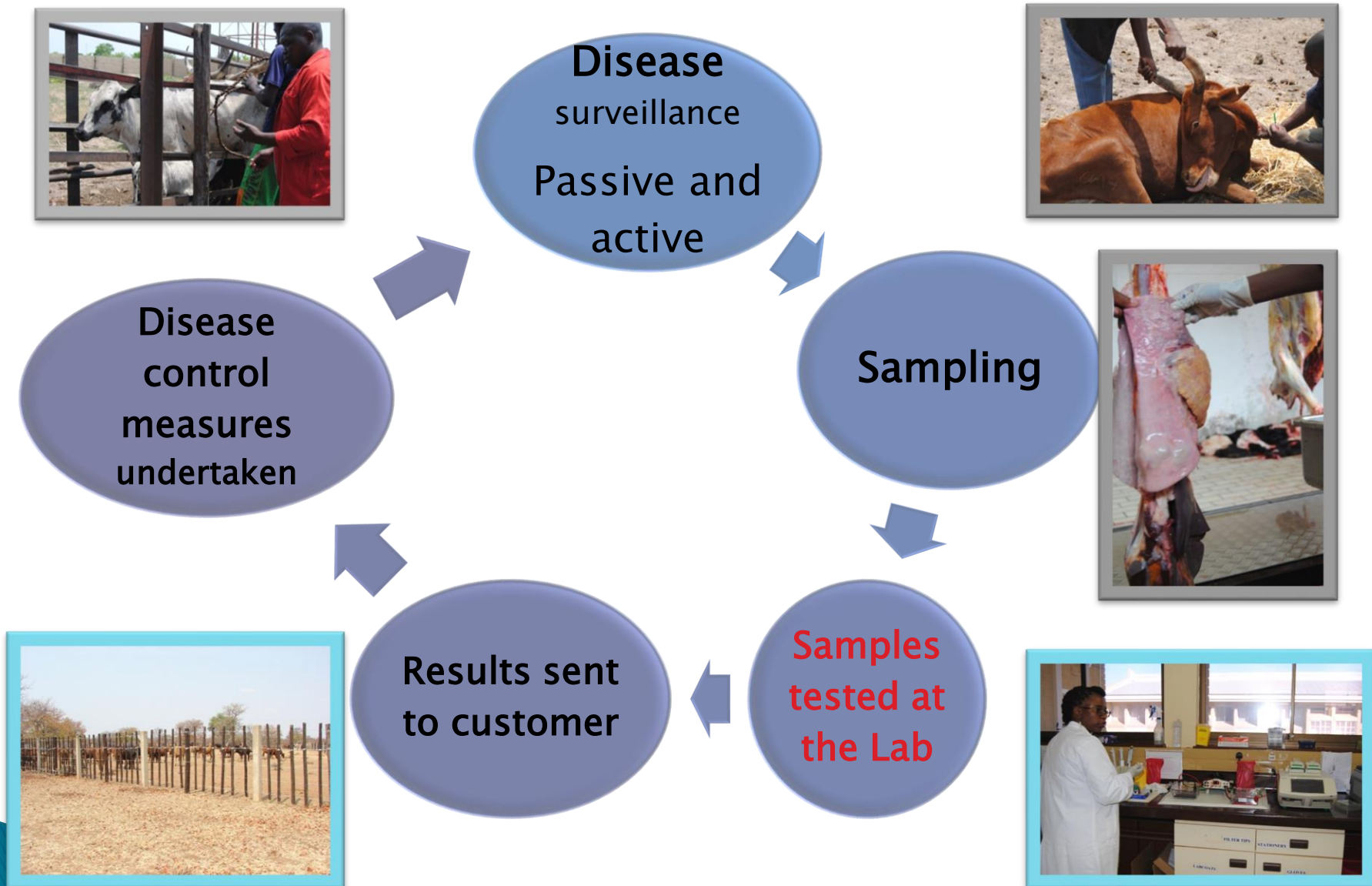
- ▶ Introduction – CBPP diagnostic tests
- ▶ Challenges with the tests
- ▶ Mandate of an WOAHA Reference labs
- ▶ What the WOAHA reference Labs can do to assist
- ▶ Conclusion
- ▶ Acknowledgements

# Contagious Bovine Pleuropneumonia (CBPP)



- ▶ CBPP is a highly contagious and infectious cattle lung disease, and is one of the most serious transboundary animal diseases in Africa
- ▶ CBPP is caused by a bacterium called *Mycoplasma mycoides* subsp *mycoides*
- ▶ CBPP is widespread in Africa, and regionally occurs **Namibia**, **Zambia** and **Angola**

# CBPP surveillance



# CBPP diagnostic tests

## 1. Tests for detection of *Mmm*

### ▶ *Culture and isolation*

*Biochemical followed by Immunochemical tests*

### ▶ *Molecular identification and typing – PCR and genotyping*

## 2. Tests for detection of immune response

Complement fixation test (CFT), competitive Enzyme Linked Immunosorbent Assay (cELISA), Immunoblotting test (IBT)


# Challenges with Isolation and PCR

- ▶ **Good quality samples** needed
- ▶ **False negatives** with isolation – presence varies with the stage of development of the lesions

And in animals treated with antibiotics

- ▶ Biochemical immunochemical tests –**laborious**
- ▶ Labs use PCR tests that are more rapid, sensitive, specific and easy to use
- ▶ Challenges of **contamination** with PCR tests

# Challenges with serological tests

- ▶ Lack **sensitivity** (false negatives) and **specificity** (false positives)
  - ▶ CFT laborious and difficult to execute
  - ▶ Difficult to source **critical reagents** like **antigen**, **positive** and **negative sera**
  - ▶ Lack of and expensive **Proficiency testing** for quality assurance and test accreditation
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# Serological tests

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Test	Sensitivity	Specificity	Advantages	Disadvantages
CFT	63.8%	98%	Can detect all sick animals with acute lesions	Can miss small proportion of animals in the early stages or animals with chronic lesions
cELISA	Same as CFT	99.8%	Can detect antibodies for longer periods	
			Easier to perform	
			Can be obtained commercially	<b>Critical shortage of kits currently</b>
IBT	Same	More specific	Used where there are doubtful CFT positives	Laborious Difficult to standardise



# Recommended CBPP tests

## Purpose

Method	Population freedom from infection	Individual animal freedom from infection prior to movement	Contribution to eradication policies	Confirmation of clinical cases	Prevalence of infection surveillance	Immune status in individual animals or populations post vaccination*
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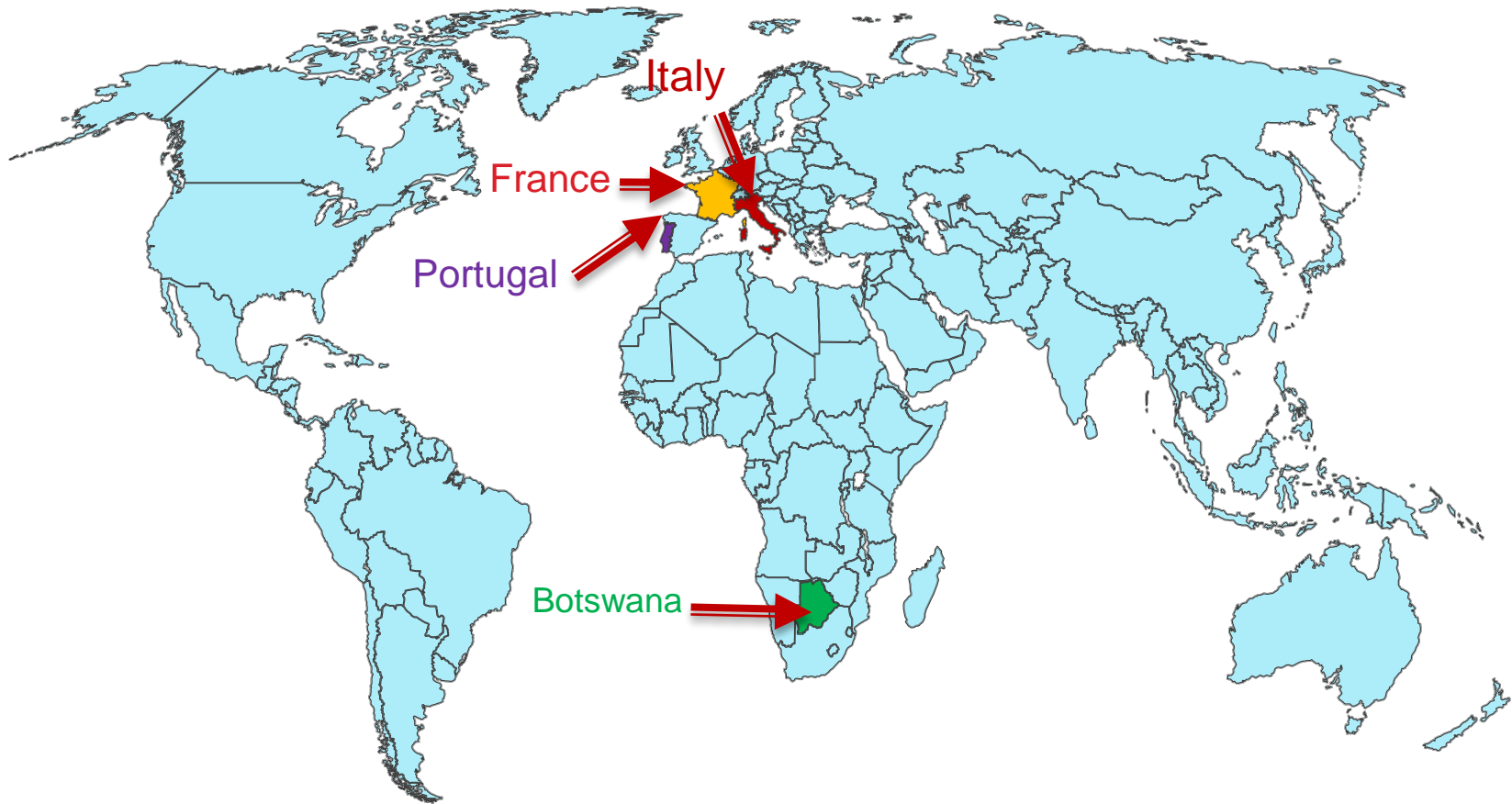
## Detection of the agent (*Mmm*)

In-vitro culture isolation (followed by species identification tests)	+	-	-	+++	-	-
Direct molecular test (PCR)	-	-	-	++	-	-

## Detection of immune response

CFT	+++	++	+++	++	+++	-
Immunoblotting	++++	++	++	++	++	-
C-ELISA	+++	++	+++	++	+++	-

# What WOAAH reference labs can do to help



## Mandate of WOAHA Reference labs

- ▶ To use, promote and disseminate **diagnostic methods validated** according to WOAHA Standards
- ▶ To recommend the prescribed and alternative tests or vaccines as WOAHA Standards
- ▶ To **develop reference material** in accordance with WOAHA requirements, implement and promote the application of WOAHA Standards



## Mandate of an WOAHA ref lab

- ▶ To carry out and/or **coordinate scientific and technical studies** in **collaboration** with other laboratories, centres or organizations
- ▶ To collect, process, analyse, publish and disseminate epizootiological data relevant to CBPP
- ▶ To provide **scientific and technical training** for personnel from WOAHA Member Countries



## Mandate of CBPP ref lab

- ▶ To maintain a system of **quality assurance**, **biosafety** and **biosecurity** relevant to CBPP
- ▶ To **organize** and **participate** in **scientific meetings** on behalf of the WOAHA
- ▶ To **establish** and **maintain** a network with other **WOAHA Reference Laboratories for CBPP** and **organize regular inter-laboratory proficiency testing** to ensure comparability of results



## Mandate of WOAHA Ref lab

- ▶ To **store** and **distribute** to national laboratories **biological reference products** and any other reagents used in the diagnosis and control of CBPP
- ▶ To **develop, standardize and validate** according WOAHA Standards new procedures for diagnosis and control of CBPP



## Mandate of WOAHA ref lab

- ▶ To organize **inter-laboratory proficiency testing** with laboratories for CBPP to ensure equivalence of results
- ▶ To place **expert consultants** at the disposal of the WOAHA



# Mandate of WOAAH Ref Lab

- ▶ To provide **diagnostic testing facilities**, and, where appropriate, **scientific** and **technical advice** on CBPP control measures to WOAAH Member Countries
- ▶ On an annual bases WOAAH reference Labs submit a report to indicating the progress made with regards to implementation of the terms of reference





# BNVL as an WOAHA ref lab

- ▶ BNVL became an WOAHA ref lab for CBPP in **2012** after a successful twinning project with **IZS**
- ▶ This has brought the expertise and provision of scientific support to the region
- ▶ As an WOAHA ref lab BNVL is mandated to carry out certain activities to assist member countries





## Role of BNVL on CBPP in the region

- ▶ What BNVL has been doing

# Coordinating regional networks

- ▶ BNVL is Coordinating a regional Scientific network on CBPP:
- ▶ Angola, Botswana, Namibia, Tanzania, Ethiopia and Zambia
- ▶ Sponsored by **AU-IBAR** and **ERFAN**
- ▶ In collaboration with **IZS**





# CBPP annual meeting



CBPP meeting Namibia  
2015

Participants 2015  
Namibia

# Achievements

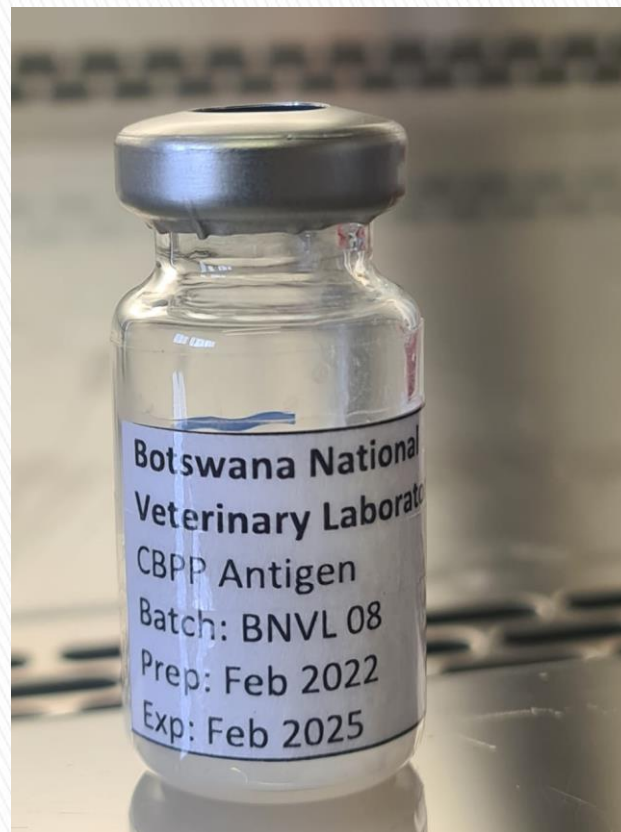
Activity	Date	Venue
Start up meeting and development of SOPs	November 2014	Livingstone – Zambia
Training on CBPP tests	May 2015	BNVL
Setting up of tests in Angola	May 2015	Humpata– Angola
Exchange of scientific personnel (recognition of clinical CBPP)	September 2015	CVRI – Zambia
Organisation of ring trials	September 2015	BNVL
Testing for CBPP surveillance	All year	Member countries
Final meeting of project	November 2015	Namibia
Production of a Good Practice Paper	February 2016	Gaborone
CBPP control strategy	June 2016	Angola

# To use, promote and disseminate diagnostic methods **validated** according to WOAHA Standards – BNVL

- ▶ Agent detection: *Mmm* **isolation** and detection by **Polymerase Chain Reaction** (PCR)
- ▶ Antibody detection: **Complement Fixation Test (CFT)** and cELISA
- ▶ **These tests have been standardised for the subregion in 2013**

# Production of reagents

- ▶ The main reagent produced is CBPP **CFT antigen**
- ▶ This is **produced, standardised and delivered** by WOAAH reference labs

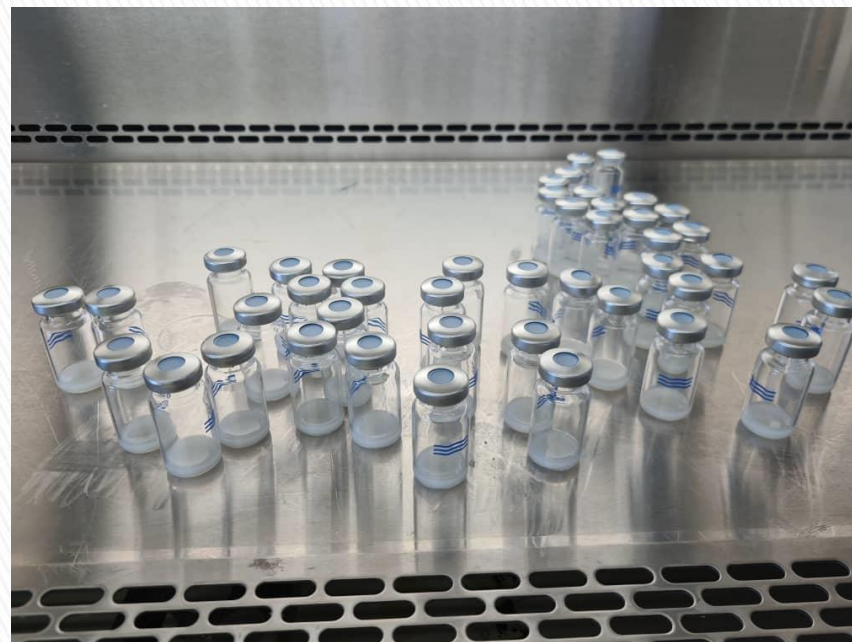


CBPP CFT antigen



## Production of reagents

- ▶ Freeze dried
- ▶ Quality assured
- ▶ Supplied CBPP CFT antigen to CVRI – Zambia, CVL– Namibia, OVI – South Africa and Humpata Lab in Angola



Distribution of antigen

CBPP CFT antigen



## Provision of diagnostic testing

- ▶ BNVL has carried out diagnostic testing for Countries in the region
- ▶ CFT for CBPP done on serum samples from Namibia, Eswatini and Zambia
- ▶ Isolation of *Mmm* for Angola

## Provision of technical training

- ▶ In September 2011 BNVL visited CVRI in **Zambia**, demonstrated CFT and quality assurance, test accredited in 2017
- ▶ In May 2015 BNVL visited The Humpata Lab in Lubango, **Angola**, assisted in setting up CFT and isolation for *Mmm*
- ▶ BNVL has trained technicians from **Uganda, Malawi, Mali, Mozambique, Ethiopia, Lesotho, Tanzania, Namibia, Angola, Zambia, Nigeria**

# Epidemiological surveillance training in Angola – 2015



Botswana and Angola  
vets sampling in Angola

Sampling in Angola

# Setting up of tests in Angola – 2015



Bench training on **CFT** and **cELISA** by BNVL



Bench training on **isolation of *Mmm*** by BNVL

# Bench training on CBPP tests at BNVL 2015



Participants from Namibia,  
Angola, Zambia, Kenya



BNVL has trained technicians  
from Uganda, Malawi, Mali,  
Mozambique, Ethiopia, Lesotho  
through IAEA sponsorship (2 –3  
months)

# Training on CBPP pathology in Zambia August 2015



Participants 2015



Training on recognition of CBPP lesions

# CBPP pathology training – Zambia







## Organisation of ring trials

**BNVL has been organising inter-laboratory ring trials with regional labs since 2007**

▶ Tests being **CFT** and **PCR**

Participating Labs are **BNVL**, **CVRI-Zambia**, **CVL-Namibia**, **OVI – South africa**,

*The purpose of which is to determine regional Labs' **capability** to conduct CBPP diagnostic tests (CFT and PCR) and to ensure **equivalence** of results*



# Aims of the ring trials & ILC

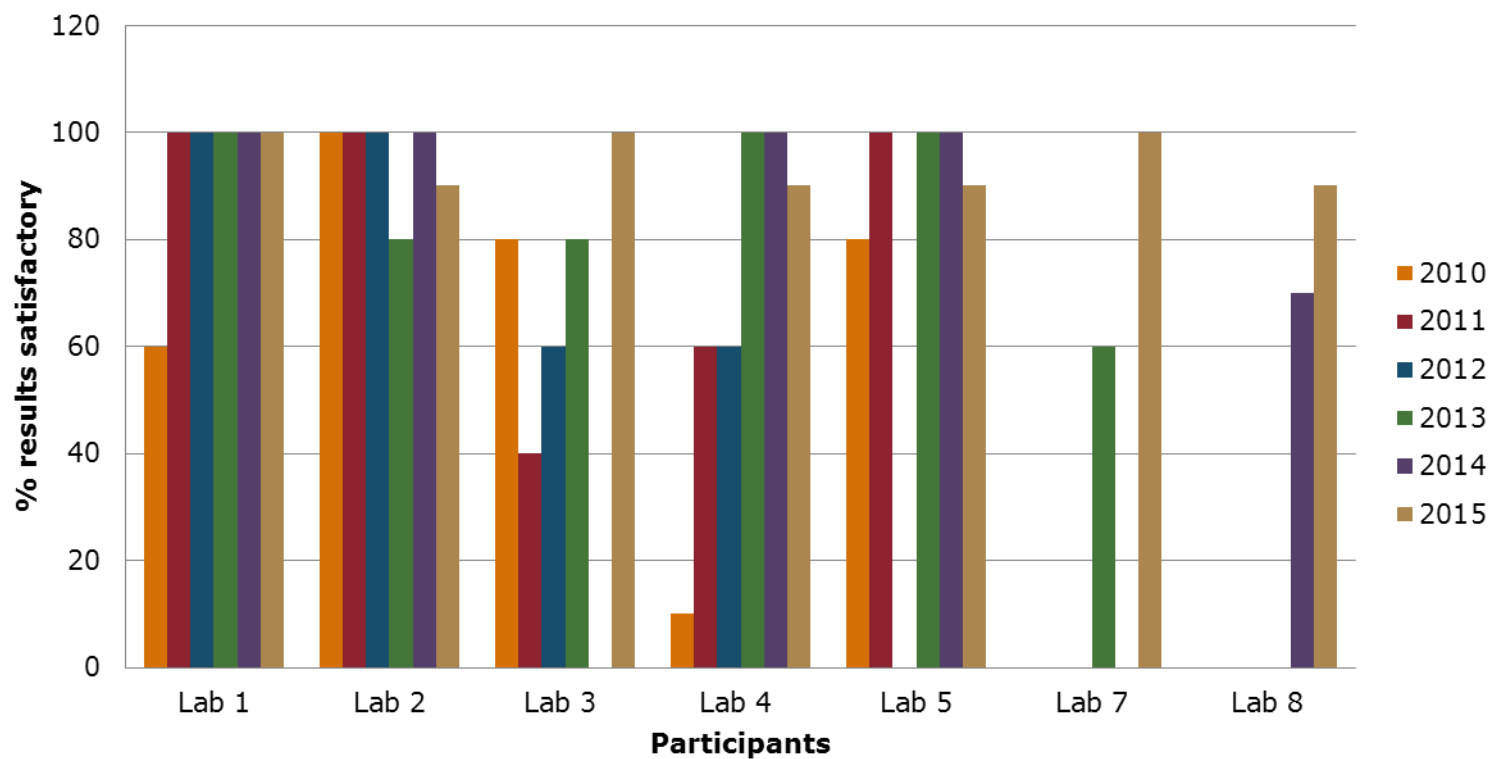
- ▶ To monitor the technical expertise of laboratories testing for CBPP
- ▶ An important quality control element & part of lab quality assurance programme
  - Assess own capabilities
  - To check performance of analysts
  - Ensure comparability of results

# Ring trials conducted

Year	Test	Distribution	Participants (number of labs)
2010	CFT	1 <sup>st</sup>	BNVL, IZS, CVL-NAM, OVI & CVRI (5)
2011	CFT PCR	2 <sup>nd</sup> 1 <sup>st</sup>	BNVL, IZS, CVL-NAM, OVI & CVRI (5)
2012	CFT	3 <sup>rd</sup>	BNVL, IZS, CVL-NAM, OVI & CVRI (5)
2013	CFT PCR	4 <sup>th</sup> 2 <sup>nd</sup>	BNVL, IZS, CIRAD, CVL, OVI & CVRI (6)
2014	CFT PCR	5 <sup>th</sup> 3 <sup>rd</sup>	BNVL, IZS, CVL, OVI, CVRI, MoALF (6)
2015	CFT PCR	6 <sup>th</sup> 4 <sup>th</sup>	BNVL, IZS, CVL, OVI, CVRI, MoALF, INIAV, ISRAILNERV, LCH, CVL-ZIM & LANAVET (11)
2022	CFT	7 <sup>th</sup>	<b>BNVL, CVL, CVRI, OVI, IZS (5)</b>

# CFT results

**Table showing satisfactory results from 2010 to 2015**



# Conclusion

- ▶ **Technical capacities** of the different laboratories can be **strengthened** through the various **trainings** conducted by WOH Ref labs
- ▶ Use of WOH **protocols**, **antigen** and **positive reference sera** for *quality assurance* of CBPP diagnostic tests
- ▶ The **ring trials** conducted after the use of **harmonised protocols** and **trainings** showed satisfactory results, this indicating the **labs capabilities for CBPP diagnosis have improved**.
- ▶ The **outcome** of this would be **improved CBPP diagnostic capacity and control** in the region.



# Conclusion

- ▶ BNVL as a WOAHP reference lab can assist the regional labs to ensure early detection and warning systems for effective CBPP control
- ▶ I would like to encourage the regional labs to continue working with BNVL in their fight against CBPP, as the fight is for all of us.
- ▶ Eradication and control of CBPP would increase animal productivity

# Food security – Zero hunger



UN Sustainable  
Development goal 2

Food for all



# Acknowledgement

I would like to thank the following:

- ▶ **WOAH** for the **twinning project** which resulted in a CBPP ref lab in the region.
- ▶ The governments of **Botswana**, **Angola**, **Namibia**, **Zambia** for their concerted effort in the fight against CBPP.
- ▶ Our Technical partner **IZS** for it's support and mentorship.
- ▶ **Everybody here for listening**



**THANK YOU**

