



LA RECHERCHE AGRONOMIQUE
POUR LE DÉVELOPPEMENT

Support from OIE PPR
Reference Laboratory to
countries

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*Regional training workshop on the OIE procedure
for the official status recognition and endorsement
of national official control programme with regard to
peste des petits ruminants (PPR) for targeted*

African countries

11 -13 June 2019

Nairobi, Kenya

Introduction



Role of the laboratory

Current diagnostic tests

Laboratory networking

Conclusion



Introduction

Control and prevention of peste des petits ruminants depends on:

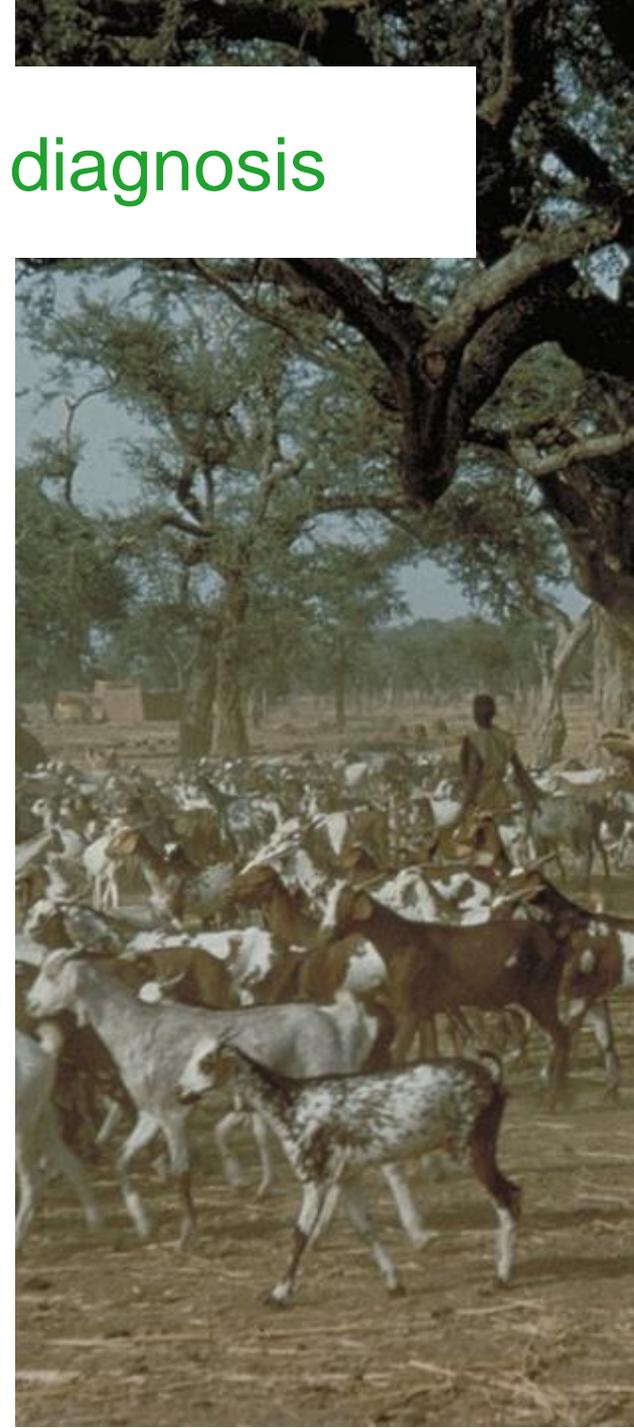
- Capacity to sample, detect and confirm disease at the earliest possible time for the design of appropriate response measures
- Diagnostic measures in place at national level should ideally combine with networks to allow for a regional approach in PPR management.

Role of the laboratory in PPR diagnosis

Laboratory confirmation of clinical cases of PPR is compulsory .

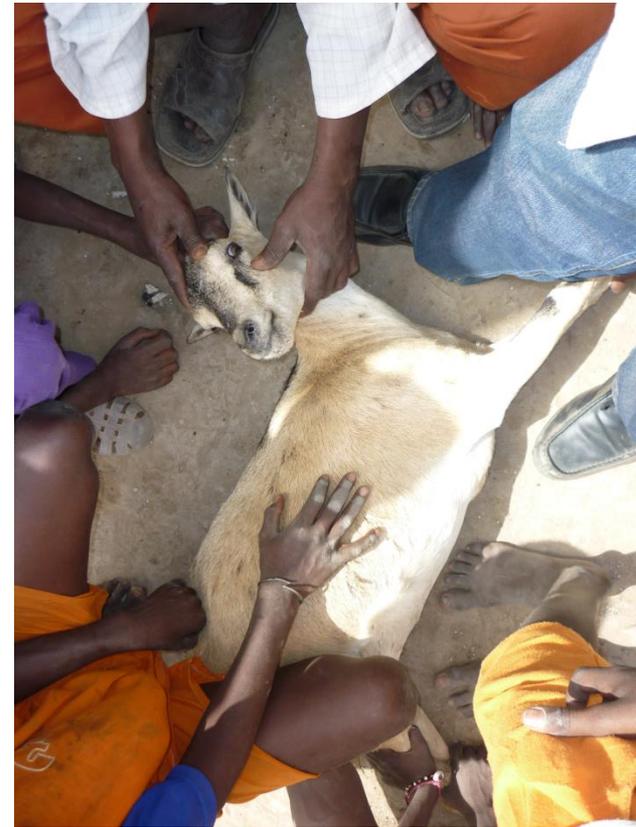
PPR can be easily confused with other diseases such as:

- Bluetongue, CCPP, Pasteurellosis,
- Definitive diagnosis of PPR is demonstrated when laboratory results are combined with clinical observations and epidemiological data.
- Essential that diagnosis whether for virology, serology, rely on validated, sensitive and specific tools



Role of the national laboratory

- Establish virological/serological diagnosis to complete observations of clinical symptoms;
- Implement quality diagnosis with standardised methods to deliver reliable PPR diagnosis results;
- Share information and expertise with veterinary services on:
 - Epidemiology of the disease,
 - Surveillance activities whether clinical, serological or virological
 - Sampling workplans, allowing to estimate disease circulation



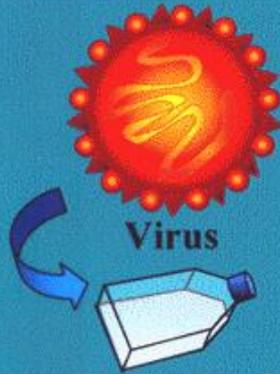
Role of the reference laboratory

- Plays also a role in the development of diagnostic tools to
 - improve sensitivity/specificity
 - speed up the lab process
- Develop tests easily transposable to laboratories with low resources
- Innovative developments to ease field samples testing and sampling (non invasive)
- Organizing or participating to international proficiency testing. Make lab networks



LABORATORY DIAGNOSIS

ISOLATION



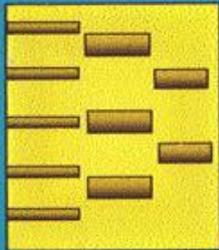
DIRECT IDENTIFICATION



Antigen

Gene

CHARACTERIZATION



Field



RETROSPECTIVE DIAGNOSIS



Antibody

CONVALESCENTS

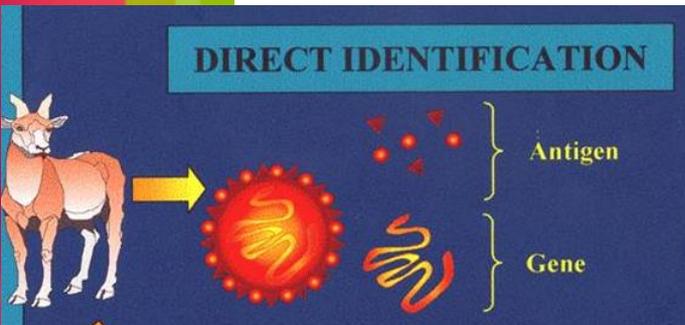
VACCINATED



SEROSURVEILLANCE

EFFICACY OF
VACCINATION
CAMPAIGN

Current laboratory tests

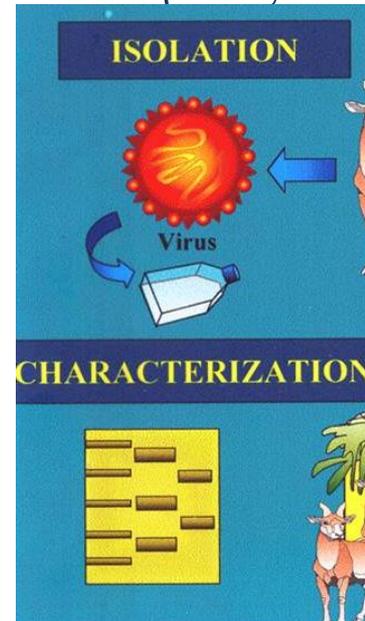


■ Virology tests : **ANTIGEN** and **GENE** detection

- ✓ Antigen Capture Elisa; Lateral Flow Device (LFD ; field test)
- ✓ Conventional RT-PCR
- ✓ Real-time RT-PCR
- ✓ LAMP PCR (field test)

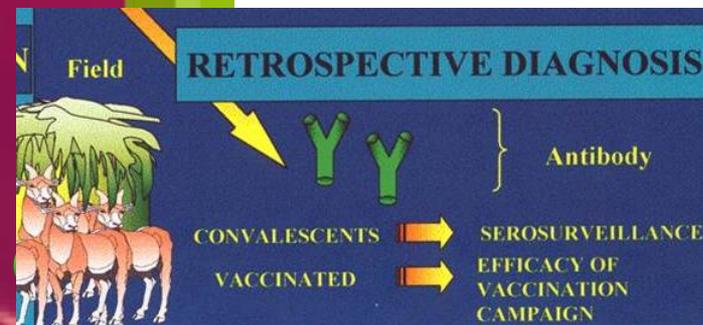
VIRUS

- ✓ Isolation on Vero cells
- ✓ Isolation on Vero Slam cells
- ✓ Characterization by sequencing



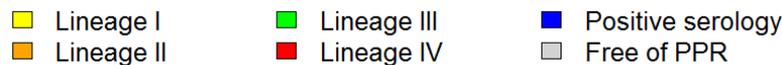
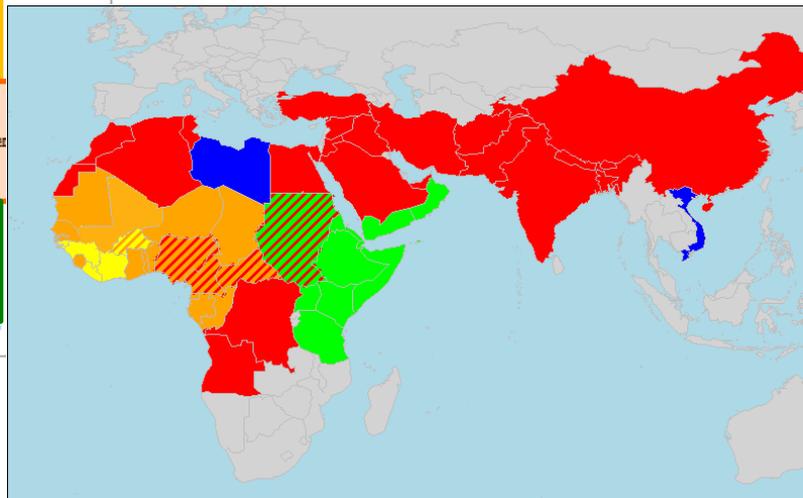
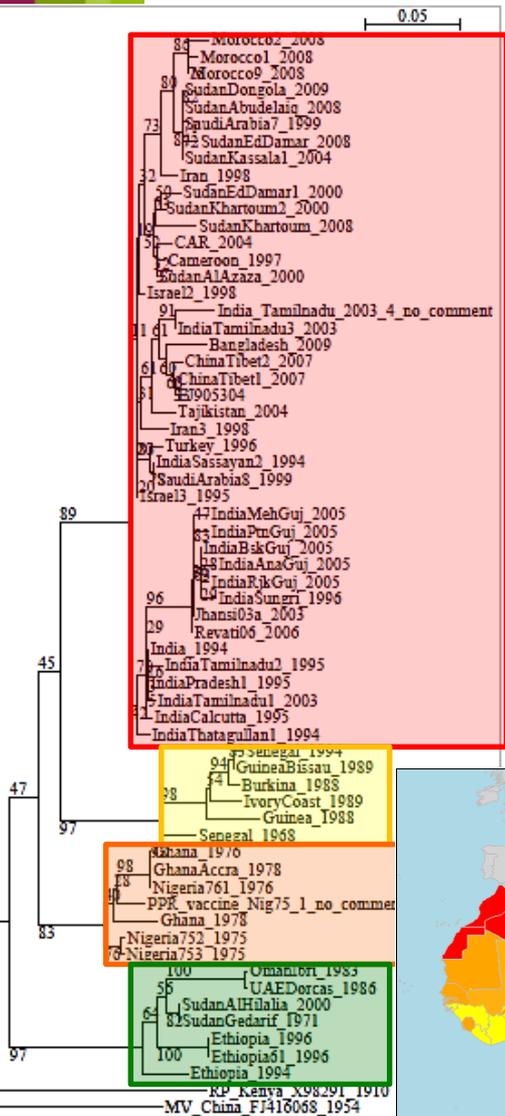
✓ Serology tests : **ANTIBODY** detection

- ✓ VNT (OIE prescribed test for international trade)
- ✓ c-Elisa,

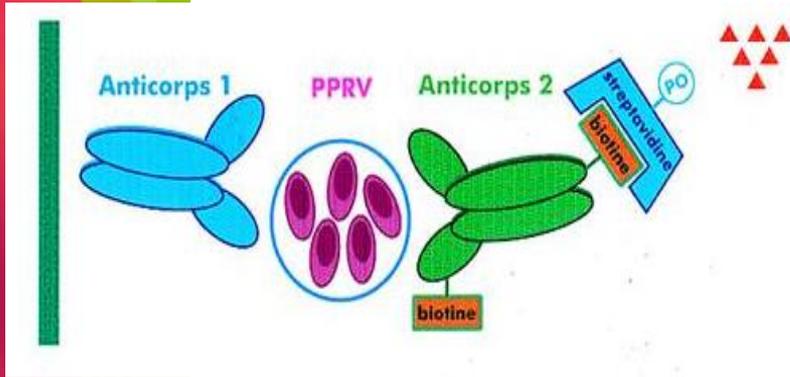


Virology tests

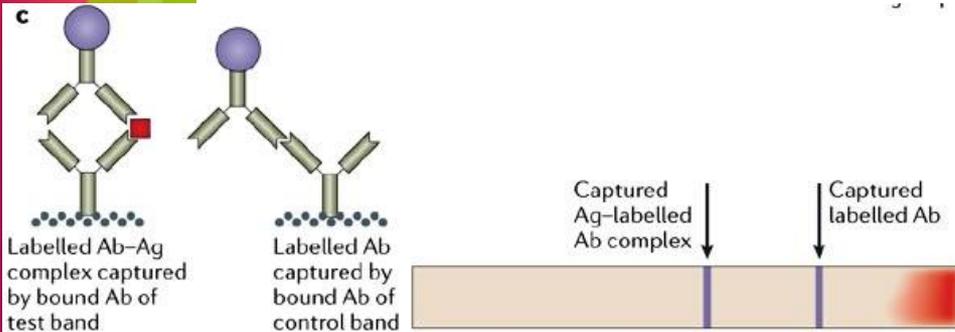
- It is crucial that laboratories use efficient tools sensitive, specific, allowing the early detection of PPR emergence/re-emergence and eventually conclude on the origin of the virus.
- Conventional RT-PCR, now widely implemented in labs, allows direct sequencing and thus for the genotyping of strains.
- With recent technical breakthroughs on NGS, diversity of field strains is established, facilitating source tracking and understanding disease diffusion pathways.



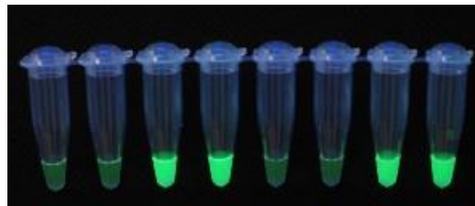
Virology tests



LFD pen-side tests



Bst: *Bacillus stearothermophilus*



- █ Oppositely, in many laboratories or resource limited, simple, rapid and robust assays can be adopted as routine techniques, able to detect viral:

- ✓ **Antigen:** such as Antigen Capture Elisa,

Sensitivity = RTPCR

- ✓ Pen-side tests: LFD

- ✓ **Gene:** LAMP-PCR: RT- loop-mediated isothermal amplification at 63° C: obtained 60min, observed by the naked eye

Sensitivity = Q-RTPCR
= 10-fold higher than conventional RT-PCR

Virology tests



- Feld tests (pen side tests)
 - Used on live animals (ocular or nasal dejection)
 - Result within minutes
- Lateral flow device (LFD test, BDSL)
 - Like pregnancy test kits
 - Sensitivity < Antigen Capture ELISA
- Pen side strip tests (IDvet)
 - Sensitivity = Antigen Capture ELISA
 - Allow recovery of RNA

Negative sample	Weak positive sample	Positive sample
 <p>Control line</p>	 <p>Control line</p>	 <p>Control line</p>



Serology tests



ELISA (developed 30 years ago)

- These tests are able to promptly detect new outbreaks of PPRV and to produce data on the prevalence in infected areas.

- A set of ELISAs were developed.

- ✓ Competitive ELISA (C-ELISA) are H or N-Mab-based, high degree of correlation to the VNT, the gold standard assay.

Well adapted (96 wells format) to large scale serology studies

OIE manual: Purpose of the methods

Method	Purpose					
	Target	<u>Confirmation of clinical cases</u>	<u>Population freedom from infection</u>	<u>Individual freedom from infection</u>	Prevalence of infection – <u>Surveillance</u>	Immune status in individual animals – <u>Vaccination</u>
ICE- ELISA	Protein	+++				
RT-PCR	Gene	+++				
QRT-PCR	Gene	+++				
Virus isolation	Virus	++				
VNT	Antibody		+++	+++	+++	+++
C-ELISA	Antibody		++	++	+++	+++

Key: +++ = recommended method;
 ++ = suitable method;

Source: Last version Chapter 3.7.9. – Peste des petits ruminants
http://www.oie.int/fileadmin/Home/fr/Health_standards/tahm/3.07.09_PPR.pdf

Veterinary laboratory networking: Examples of laboratory networks. Main activities of these networks.

In Africa, networks of laboratories were established with specific activities on PPR with the close involvement of main reference laboratories (ex RESOLAB...).

A European network of 28 NRL has been instituted under the auspices of the EU, creation of EURL-PPR

The duty of the EURL- PPR (CIRAD) is to collaborate with NRL notably on:

- Diagnostic techniques harmonization by implementing PT.
- Implementation of quality assurance and biosafety, biosecurity practices;
- Technical training in diagnostic techniques;
- Support for diagnosis;
- Sharing sampling workplans, field expertise with veterinary services.

RESOLAB
SOUS RÉSEAU RAGE

RESOLAB
SOUS RÉSEAU PPR

RESOLAB
SOUS RÉSEAU FMD

RESECOP
FRAÏRES ANIMALES

RESEPI
EPIDEMIO-SURVEILLANCE

RESOLAB

IDENTIFY



Veterinary laboratory networking: OIE Reference Laboratories

- The OIE Reference laboratories (3 at present in the world for PPR) plays an important role in assisting laboratories from OIE country members, supporting the training and diagnostic activities, giving opportunities to:
 - ✓ Participate to ring trials,
 - ✓ Involved in OIE twinning projects,
- Supplying reagents, scientific and technical knowledge.
- Finally support to GCES for PPR through a worldwide network of OIE laboratories.

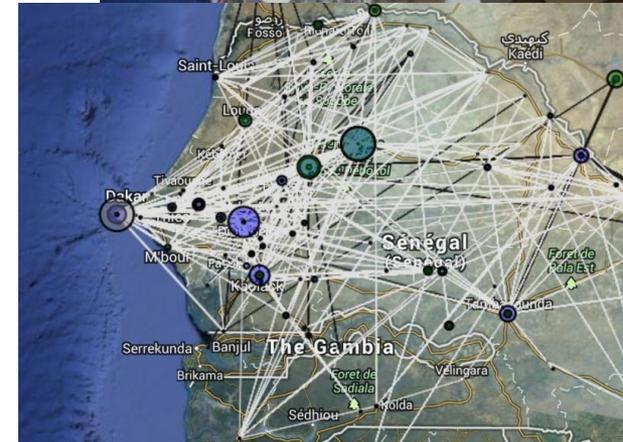
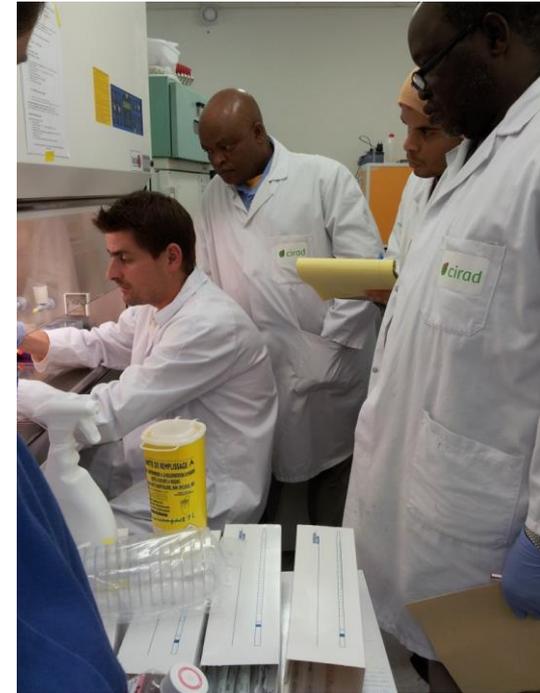


Twinning: strengthened diagnostic / quality assurance, + strengthened disease surveillance capabilities adapted to the specific epidemiological situation in the country.

Connect the main research institutes and laboratories throughout the world having experience in the study of PPR, including the 3 OIE Reference Laboratories for PPR.

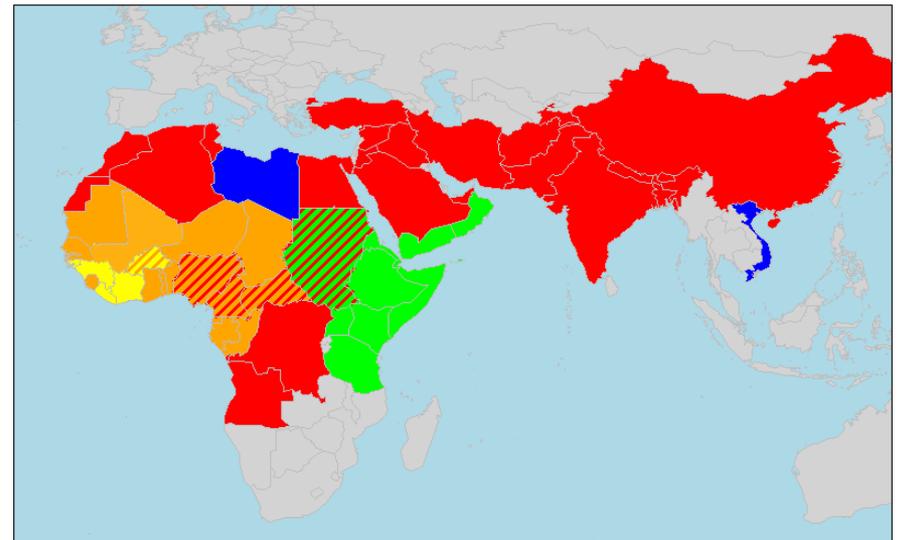
Conclusion 1/2

- It is crucial that laboratories implement efficient diagnostics allowing the early detection of PPR.
- All these tests will allow to appreciate at national level:
 - ✓ the presence/spreading of the disease into new areas or to certify freedom from the disease.



Conclusion 2/2

- It is important to integrate national/regional laboratories activities and epidemiological surveillance networks.
- Allow to :
 - ✓ Clarify the regional situation of peste des petits ruminants and understand PPRV diffusion pathway,
 - ✓ Map the health risk areas to improve the coordination of prevention and control measures.



■ Lineage I
■ Lineage II

■ Lineage III
■ Lineage IV

■ Positive serology
■ Free of PPR

Team involved



Arnaud Bataille

PPR expertise, evolutionist,
reverse genetics methodology



Samia Guendouz

PPR expertise, NGS methodology



Renata Servan de Almeida
siRNA methodology, animal
challenges

Geneviève Libeau
PPR expertise, ELISA and
vaccine development



Olivier Kwiatek
PPR expertise, diagnosis tools
development



Tetiana Kwan Tat
Intellectual Property



CIRAD (ASTRE, UMR117)

Thank you



Thank you

