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DEVELOPMENT AND EVALUATION OF A MULTIPLEX CONVENTIONAL RT-PCR FOR SIMULTANEOUS DETECTION AND TYPING OF FMDV IN WEST AFRICA

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anses — French agency for food, environmental and occupational health & safety — Investigate, evaluate, protect

eufmd — european commission for the control of foot-and-mouth disease

OS18 | 29/31 OCT 2018 | BORGO EGNAZIA , Puglia, Italy

CONTEXT

West Africa : circulation of O/A/SAT1/SAT2 types ▸Pool 5

https://www.wealthresult.com/buying-and-selling/selling-goat-nigeria https://africafeeds.com/wp-content/uploads/2017/07/pigs-in-Africa.jpg https://assets.irinnews.org/styles/responsive_large/s3/cows_2.jpg

http://www.africaranking.com/wp-content/uploads/2014/12/West-African-Countries.jpg

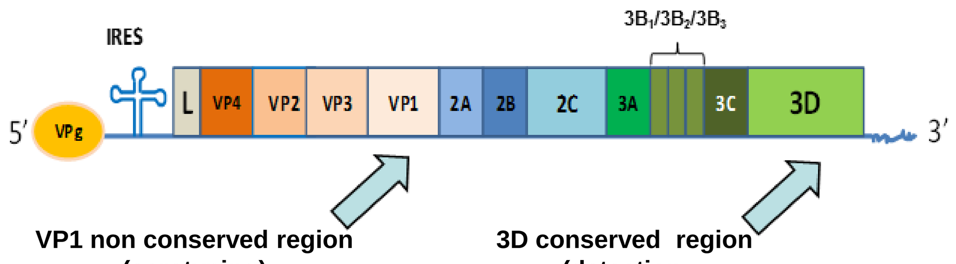
- ✓**FMD is endemic in West Africa:**
  - ▸*Continuous FMDV circulation*
- ✓**Lack of diagnostic capacities & under-reporting:**
  - ▸*Lack of information on FMD dynamics*
- ✓**Difficulties for implementation of control strategies**
  - ▸ *risk analysis of FMDV incursion/spread?*
- ✓**Towards the goal of FMD eradication…**
  - ▸*Knowledge definitely required on FMDV strains circulating in West Africa*

http://www.routard.com/photos/senegal/

AIM

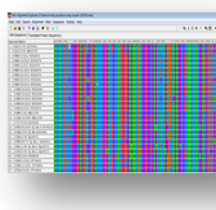
➡ **To develop and evaluate a conventional multiplex RT-PCR for both detection and typing of FMD viruses circulating in West Africa**

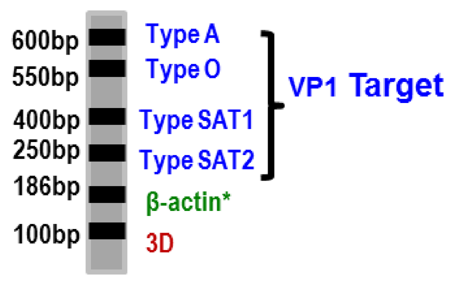
Target sequences



Genome analysis & Design of primers

WA FMDV sequence analysis





Expected Amplicon size

*B-actin housekeeping gene used as quality control of the process

Evaluation Steps

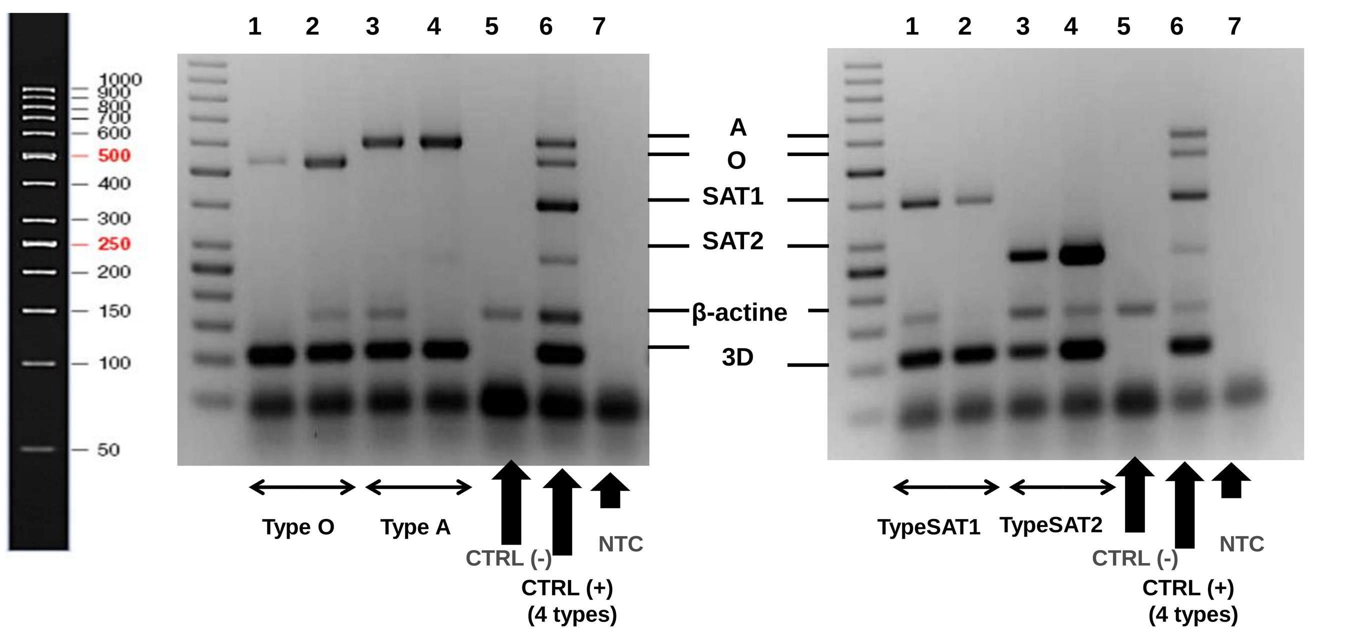
1) Simplex → 6-plex — Panel of reference strains

2) Prototype 6-plex (O, A, SAT1, SAT2, 3D, B-actine) — Field Samples and Field Isolates

Target specificity & amplicon sizes allowing easy visual interpretation of RT-PCR profiles using agarose gel electrophoresis

6 PLEX RT-PCR PROTOTYPE (O/A/SAT1/SAT2/3D/B-ACTIN)

▸ **Developped using reference strains and field isolates**



Analytical Specificity of RT-PCR 6-plex

Strain serotype	n=	3D	Typing
Type O	7	7/7	7/7
Originated from West Africa	2	2/2	2/2
Other topotypes	5	5/5	5/5
Type A	8	8/8	5/8
Originated from West Africa	2	2/2	2/2
Other topotypes	6	6/6	3/6
Type SAT1 strains (Ken/Bot)	2	2/2	2/2
Type SAT2 strains (Lib/Egy/Zim)	3	3/3	3/3
Type SAT3 strains (Zim)	1	1/1	0/1
Type C	1	1/1	0/1
Type Asia-1	2	2/2	0/2
SVDV*	3	0/3	0/3

* *Swine vesicular disease virus (i.e. other picornavirus)* (differential diagnostic of FMD)

▸ **Diagnostic sensitivity & specificity**

Diagnostic sensitivity (on positive samples type O & A)

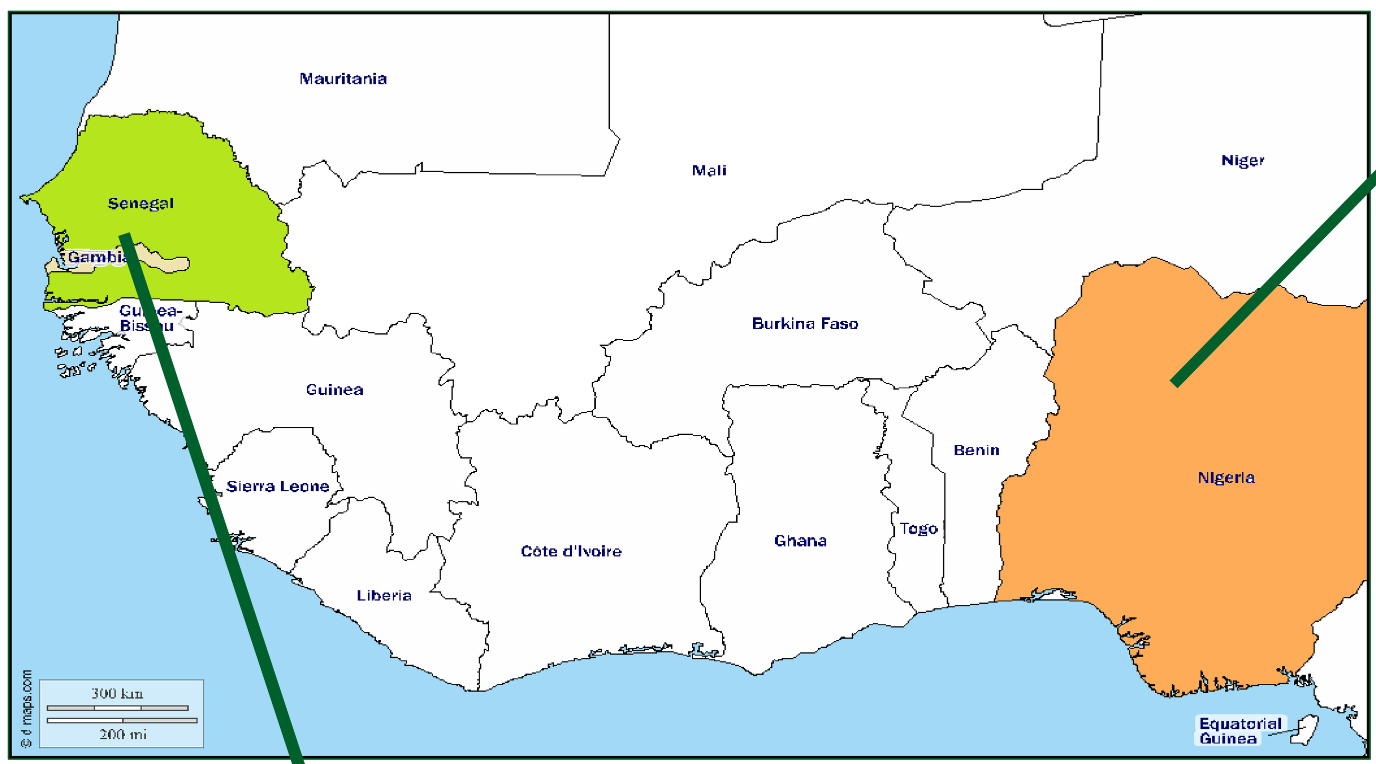
Positive field samples and the corresponding isolates (West Africa; Benin 2010)

Sample nature	Type	Real-time RT-PCR (3D Target)	RT-PCR 6-plex		RT-PCR 3-plex		Simplex (VP1 typing O or A)
			Detection (3D)	Typing (VP1 O or A)	Detection (3D)	Typing (VP1 O or A)	
Field Samples	O	28/28	28/28	8/28	28/28	13/28	28/28
Field Isolates	O	28/28	28/28	24/28	28/28	28/28	28/28
Field Samples	A	9/9	9/9	6/9	9/9	6/9	9/9
Field Isolates	A	9/9	9/9	9/9	9/9	9/9	9/9

Diagnostic specificity (negative samples)

- ✓Negative Fields samples (n=24) /Bovine, Swine, Ovine, Caprine/ (France 2013-14)
- ✓None amplification was observed for targets 3D and VP1.
- ✓B-actin amplification was observed for all samples

EVALUATION OF THIS PROTOTYPE ON FIELD SAMPLES THROUGH COLLABORATIONS IN ENDEMIC COUNTRIES



▸ **Evaluation on field samples (Senegal , ISRA/LNERV)**

- ➢ 51 samples
- ➢ Collected between 2008-2014
- ➢ From Senegal & Gambia

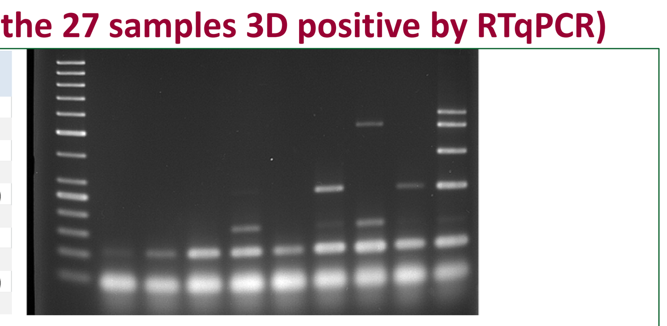
+ wellcome of a trainee (2 weeks) from LNREV

Sample treatment → RNA Extraction → Multiplex RT-PCR → Electrophoresis Interpretation

≈ 6h

Results of the RTqPCR 6-plex assay (on the 27 samples 3D positive by RTqPCR)

Sample	Well number	Pan FA (3D)	B-actin	Typing
Ladder	1	/	/	/
SEN 1	2	POS	NEG	NEG
SEN 2	3	POS	NEG	NEG
SEN 3	4	POS	NEG	NEG
SEN 4	5	POS	POS	SAT2 (low)
SEN 5	6	POS	NEG	NEG
SEN 6	7	POS	NEG	SAT2
SEN 7	8	POS	POS	O
SEN 7B	9	POS	NEG	SAT2 (low)
T+O/A/SAT1/SAT2	10	POS	POS (low)	POS



sample type	analysed	RtqPCR Duplex pan-FA		Rt-PCR 6 plex		
		3D positive	beta actin	3D positive2	beta actin 2	Typing
Epithelium sample (bovine)	15	14	15	14 (Ct<25)	9 (Ct<27)	SAT2 (n=3); O (n=3) => 6/14
Epithelium sample (swine)	2	1	2	1 (Ct=26)	0	UND
Tongue swab (bovine)	14	6	10	1 (Ct=22=	3 (Ct<30)	UND
Vesicles swab (bovine)	6	4	4	2 (Ct<24)	2 (Ct<27)	UND
Mouth swab (bovine)	2	2	2	2 (Ct=15)	1 (Ct<27)	O (n=1) => 1/2
Nasal swab (goat)	7	0	7	0	5 (Ct<27)	UND
Blood sample (bovine)	5	0	5	ND	ND	ND
Total	51	27	45	20	20	7 out of 20

- ✓**20/27 samples detected positive and 7/20 typed using 6-plex**
- ✓**Overall, 10/20 samples typed (6plex + 3plex confirmation)**

▸ **Evaluation on field samples & corresponding isolates (Nigeria, NVRI)**

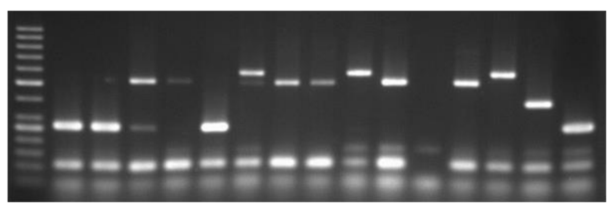
- ➢ 40 epithelium samples (38 bovine/2 ovine)
- ➢ Collected between 2011-2016
- ➢ From 6 states

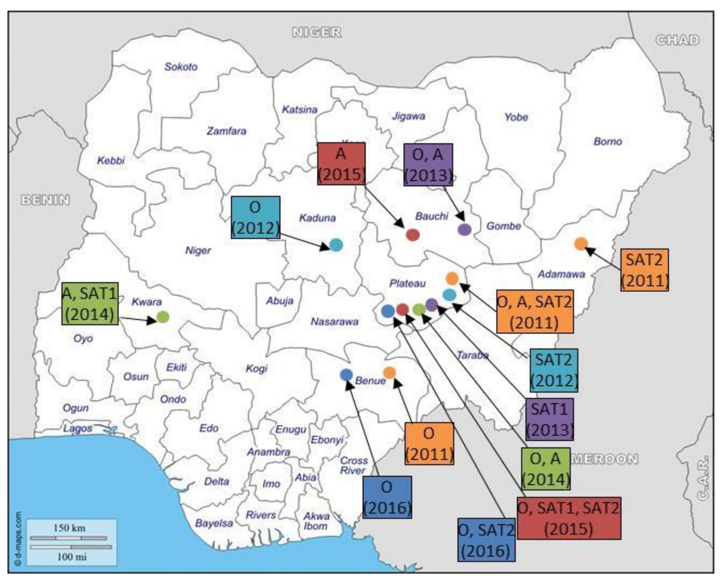
State	2011	2012	2013	2014	2015	2016	TOTAL Samples
	Year of sampling						
Adamawa	n=1 SAT2						1
Bauchi			n=4 O, A		n=2 A		6
Benue	n=2 O					n=1 O	3
Kaduna		n=1 O					1
Kwara				n=2 A, SAT1			2
Plateau	n=5 O, A, SAT2	n=2 SAT2	n=1 SAT1	n=5 O, A	n=7 O, SAT1, SAT2	n=7 O, SAT2	27

Results (6plex *vs* Ag-Elisa on isolates)

S/No	Sample ID	rtRT-PCR	VI	Ag-ELISA Serotype	Ag-ELISA OD	RT-PCR 6-plex 1st ess	RT-PCR 6-plex 2nd ess
1.	PL/BK/08196	Pos	2nd P	SAT2	0.291	SAT2	/
2.	PL/BK/08708	Pos	2nd P	SAT2	0.304	SAT2	/
3.	PL/DN/006/E	Pos	2nd P	O	1.316	O/SAT2	/
4.	PL/DN/001/E	Pos	1st P	O	0.346	O	/
5.	PL/DN/005/E	Pos	1st P	SAT2	0.333	SAT2	/
6.	BAU/T/CI/13	Pos	1st P	A	0.774	A/O	/
7.	BAU/T/C5/13	Pos	1st P	O	0.955	O	/
8.	BAU/T/A1/13	Pos	1st P	O	0.786	O	/
9.	BAU/T/C4/13	Pos	1st P	A	0.29	A	/
10.	PL/KA/06/04/A-1	Pos	1st P	O	1.137	O	/
11.	PL/KA/06/04/1B-1	Pos	1st P	O	1.217	O	/
12.	PL/KA/06/04/1B-2	Pos	1st P	O	0.954	O	/
13.	KD/KAU/01/12	Pos	1st P	O	0.868	O	/
14.	BN/G/4/16	Pos	1st P	O	1.281	O	/
15.	PL/BL/A/2/16	Pos	1st P	O	1.293	O	/
16.	PL/BL/A/1/16	Pos	1st P	O	1.249	O	/
17.	PL/BL/B/3/16	Pos	1st P	SAT2	0.523	SAT2	/
18.	PL/PKN/M/5/16	Pos	1st P	O	1.212	O low	/
19.	PL/PKN/M/2/16	Pos	1st P	O	1.169	O	/
20.	PL/JS/GE/01/16	Pos	1st P	O	0.735	O	/
21.	PL/JS/GE/02/16	Pos	1st P	O	1.420	O	/
22.	PL/KARA/15/04/2/1!	Pos	1st P	undet	/	Neg	O?
23.	PL/KARA/15/04/1/1!	Pos	2nd P	O	1.642	O	/
24.	PL/JS/KARA/12/15/4	Pos	1st P	O	1.015	O	/
25.	PL/JS/KARA/12/15/1	Pos	1st P	A low	0.169	SAT2 low	O ?/SAT2
26.	BA/TR/B/1	Pos	1st P	A low	0.162	A	/
27.	BA/TR/A/1	Pos	1st P	A low	0.179	A	/
28.	PL/TNK/04/11	Pos	1st P	A	0.610	A low	/
29.	PL/TNK/02/11	Pos	1st P	SAT 2	1.025	SAT2	/
30.	PL/796/15	Pos	1st P	undet	/	Nég	Nég
31.	PL/800/15	Pos	1st P	O	0.262	Nég	O
32	PL/372/15	Pos	1st P	undet	/	Nég	SAT1 (Low)
33.	PL/517/15	Pos	1st P	SAT1	0.226	Nég	Nég
34.	KW/1/5/14	Pos	1st P	A	0.641	Nég	/
35.	PL/BLD/02B	Pos	1st P	A	0.564	Nég	/
36.	PL/BLD/03B	Pos	1st P	A	0.211	Nég	/
37.	AD/GMB/03	Pos	1st P	SAT2	0.416	SAT2	/
38.	FMD/191/2B	Pos	1st P	undet	/	Nég	Nég
39.	BN/FMD/2011/40E	Pos	1st P	O	0.587	O	/
40.	BN/FMD/2011/40E	Pos	1st P	O	0.490	O	/

- Confirmed with 3-plex (sample 3)
- Confirmed with 3-plex (sample 6)
- Sample Typed as SAT1 with 6-plex (new primers added) (sample 22)
- Confirmed with 3-plex (sample 25)
- Sample Typed as SAT1 with 6-plex (new primers For/Rev added) (sample 30)
- Samples Typed as SAT1 with 6-plex (new primers For/Rev added) (samples 32–33)
- Samples Typed as A with 6-plex (new primers Rev added) (samples 34–36)
- Sample Typed as SAT1 with 6-plex (new primers For/Rev added) (sample 38)





- ✓**All isolates typed using 6plex (37/40) or 3plex confirmation (3/40)**
- ✓**Last results: 22 fied samples typed using 6plex (including 4 whose isolates not typed by Ag-Elisa)**

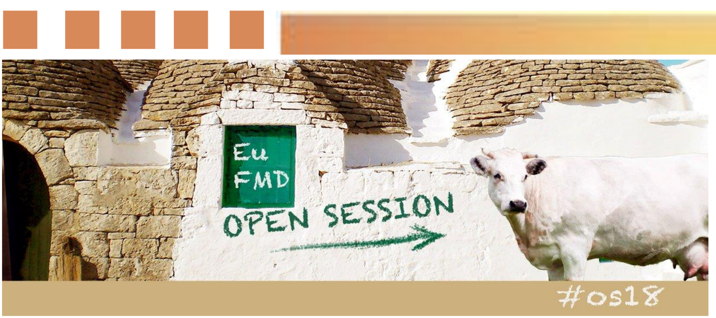
What Next

- ➢ Sequencing and optimization of SAT2 / O typing
- ➢ Test of the optimized RT-PCR on the original samples and corresponding isolates
- ➢ Evaluation of the RT-PCR at NVRI and LNERV/ISRA on field samples (in progress)

CONCLUSION &PERSPECTIVES

A PROMISING CONVENTIONAL MULTIPLEX RT-PCR METHOD

- ▸ *Allowing simultaneous detection and typing of FMD viruses*
- ✓**Evaluated on reference strains, WA field samples & corresponding isolates**
- ✓**Specific**
- ✓**Flexible (triplex, 4- plex, 5-plex, 6-plex as required)**
- ▸ *Easy to implement in diagnostic laboratories*
- ✓**No need of expensive laboratory equipment and reagents**
- ✓**Could be used for early and rapid detection**
- ✓**Currently used in Nigeria and Senegal (technological transfer and primer mixes provided)**
- ✓**Ready-to-used mastermix under study**



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