Veterinary Microbiology 171 (2014) 175–181

Contents lists available at ScienceDirect

Veterinary Microbiology

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

Short Communication

First isolation and molecular characterization of foot-and-mouth disease virus in Benin

Kamila Gorna [a,1], Evelyne Houndjè [b,c,d,1], Aurore Romey [a], Anthony Relmy [a], Sandra Blaise-Boisseau [a], Marc Kpodékon [b,e], Claude Saegerman [d], François Moutou [a], Stephan Zientara [a], Labib Bakkali Kassimi [a,*]

[a] ANSES, Laboratory for Animal Health, UMR1161 (ANSES, Inra, Enva), 23 Avenue du Général de Gaulle, 94706 Maisons-Alfort, France
[b] Laboratoire de Recherche en Biologie Appliquée, Ecole Polytechnique d'Abomey-Calavi, Université d'Abomey-Calavi, 01 BP 2009 Cotonou, Benin
[c] Département de Santé Animale, Institut de Médecine Tropicale, Nationalestraat, 155, Anvers, Belgium
[d] Research Unit of Epidemiology and Risk Analysis applied to veterinary science (UREAR-ULg), Fundamental and Applied Research for Animals & Health, Faculty of Veterinary Medicine, University of Liege, Boulevard de Colonster, 20, B42, 4000 Liege, Belgium
[e] Département de Production et Santé Animales, Ecole Polytechnique d'Abomey-Calavi, Université d'Abomey-Calavi, 01 BP 2009 Cotonou, Benin

ARTICLE INFO

Article history:
Received 27 September 2013
Received in revised form 25 February 2014
Accepted 3 March 2014

Keywords:
Foot-and-mouth disease
Virus isolation
West Africa
Benin

ABSTRACT

Foot-and-mouth disease (FMD) is a highly contagious viral disease of cloven-hoofed animals. It is one of the most economically devastating diseases affecting livestock animals. In West Africa, where constant circulation of FMD virus (FMDV) is assumed, very few studies on the characterization of circulating strains have been published. This study describes the first isolation and characterization of FMDV in Benin. FMDV was isolated from 42 samples. Antigen Capture Elisa (Ag-ELISA) and VP1 coding sequence analysis revealed 33 strains of serotype O and 9 strains of serotype A. Phylogenetic analysis of the VP1 sequence revealed two different groups of type O isolates and one group of A isolates. VP1 sequence comparison with the sequences available in the GenBank database revealed a close relationship of the Benin isolates with topotype O of West Africa and with African topotype A of genotype VI. Knowledge of the recent strains circulating in Benin should contribute to better selection of vaccine strains and enable the updating of molecular epidemiology data available for West Africa in general.

1. Introduction

Foot-and-mouth disease virus (FMDV) is a member of the *Aphthovirus* genus within the *Picornaviridae* family. There are seven immunologically distinct serotypes, namely O, A, C, Asia 1, SAT 1, SAT 2 and SAT 3. This virus is responsible for highly contagious trans-boundary disease of cloven-hoofed domestic and wild animals (Grubman and Baxt, 2004). Foot-and-mouth disease (FMD) is characterized by fever and vesicles or blisters that appear on the mouth, feet, teats, and between the hooves. Many affected animals recover, but the disease leaves them debilitated and causes severe losses in the production of meat and milk. Due to measures employed to control outbreaks and losses resulting from embargos on trade of animals and animal products, FMD is one of the most economically devastating diseases of livestock (Thompson et al., 2002).

The problem of the occurrence of FMDV in West Africa remains complex and is of concern. Six of the seven FMDV

* Corresponding author. Tel.: +33 149771317; fax: +33 143689762.
E-mail address: Labib.bakkali-kassimi@anses.fr (L. Bakkali Kassimi).
[1] These authors contributed equally to the work.

http://dx.doi.org/10.1016/j.vetmic.2014.03.003
0378-1135/

serotypes (excluding Asia 1) have already been reported in Africa where O, A, SAT 1 and SAT 2 serotypes are especially widespread and occur regularly in West and sub-Saharan region (Vosloo et al., 2002). However, very little is known about the FMDV strains circulating in West Africa and there are no recent data on the outbreaks from this region (Dehoux and Hounsou-Ve, 1991; Bastos et al., 2003; Sangare et al., 2001, 2003).

The aim of this work was to isolate and characterize FMDV from samples collected from suspected cases of FMD in Benin. Thus, we reported the first FMDV isolation in this country and its characterization. The genetic relationships between the isolated viruses and FMDV strains from other regions in Africa were analyzed. These findings are of great interest to the study of the evolution of FMD in this region of Africa and for updating currently available molecular data.

2. Materials and methods

2.1. Samples

Samples ($n = 77$) of epithelial tissue from vesicular lesions were collected between June and August 2010 from suspected FMD-infected Cattle in 3 different departments of the northern part of Benin: Borgou (52 samples), Atacora (12 samples) and Alibori (13 samples) (Fig. 1). The samples were placed in a transport medium (OIE Manual, 2012) and shipped frozen to the French Reference Laboratory for FMD in compliance with *ad hoc* biosafety international standard. After thawing, the samples were removed from the transport medium and washed in cell culture medium without Fetal Calf Serum (FCS). A 5% or 10% (w/v) suspension of each epithelial tissue sample was prepared in culture medium and grounded with sterile sand using a mortar and pestle. The suspensions were clarified by centrifugation and stored at −80 °C until further analysis.

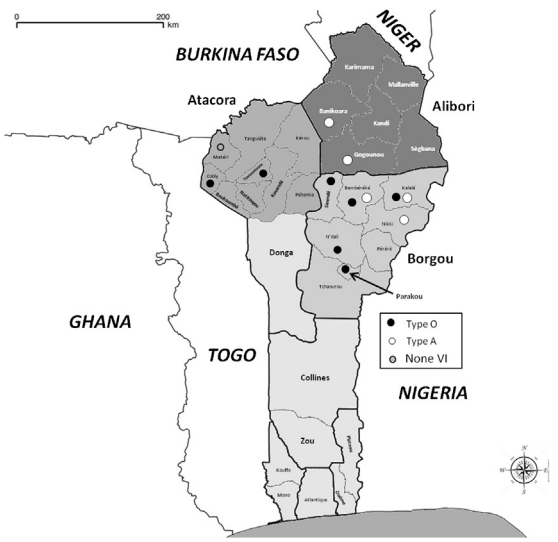

Fig. 1. Map of Benin showing the places of sample collection and the geographical location of FMDV serotypes isolated in this study. NONE VI #: None FMDV was isolated from the samples collected in that place.

2.2. Virus isolation

Virus isolation (VI) was performed on a monolayer culture of fetal goat tongue cell line (ZZ-R 127 cell line). This cell line was found to be highly sensitive to the replication of FMDV. Its sensitivity is only slightly inferior to that of primary bovine thyroid (BTY) cells, most frequently used for FMDV isolation, but significantly higher than that of IBRS-2 cells (swine kidney cell line) (Brehm et al., 2009). Cell monolayers were inoculated with pure and 2-fold diluted sample suspensions and incubated for 1 h at 37 °C. Afterwards, fresh cell culture medium without FCS was added and the cultures were incubated at 37 °C and monitored for a cytopathic effect (CPE) for 72 h. When no CPE was observed, the cells were subjected to one freeze-thaw cycle, clarified and inoculated to fresh cell monolayer. Sample was considered negative if no CPE was observed after 72 h of the second cell passage. If the CPE was observed, the viral suspension was stored at −80 °C. The isolated virus was propagated on IBRS-2 cells to produce a stock of each virus that was used for further analysis. For the samples that were found negative for VI on the ZZ-R127 cell line, the VI was also attempted by using IBRS-2 cells. However, none virus was isolated.

2.3. Ag-ELISA

Due to the insufficient volume of the sample, the indirect sandwich Ag-ELISA for FMDV typing was performed from the clarified suspension of infected IBRS-2 cells according to the protocol described in the OIE Manual for Terrestrial Animals (OIE, 2012). This method is based on methodology and reagents developed at the FMD World Reference Laboratory at Pirbright (Ferris and Dawson, 1988).

2.4. One-step duplex rtRT-PCR pan-FMDV

Virus RNA was extracted from the sample and infected cells by using silica columns (QIAamp Viral RNA mini kit, Qiagen) according to the manufacturer's instructions.

The rtRT-PCR was performed by using the Kit AgPath-ID one-step RT-PCR reagent (Life Technologies) according to the manufacturer's instructions in a total volume of 25 μl where 5 μl of RNA were added. The concentration of primers and probe were as previously described for FMDV polymerase 3D target (Callahan et al., 2002) and for β-actin target (Toussaint et al., 2007). The Pan-FMDV 3D probe was labeled by FAM and the β-actin by VIC fluorescent dye at 5′ end. The mixtures were placed in ABI 7300 thermocycler (Life Technologies) to perform the reverse transcription for 10 min at 45 °C followed by a denaturation step for 10 min at 95 °C and 45 cycles of hybridization/elongation of 15 s at 95 °C and 1 min at 60 °C.

2.5. Sequencing and sequence analysis

RT-PCR for amplification of the VP1 protein coding sequence of FMDV was performed by using the One-Step RT-PCR kit (Qiagen) according to the manufacturer's instructions. The serotype specific primers of type O, A,

Table 1
List of FMD viruses isolated from samples collected in Benin in 2010 and the GenBank accession number of VP1 sequences corresponding to the prototype of each genetic variant collected from specific geographic location.

Sample no.	Department	Commune	Isolate	Serotype	Genetic variant number	Gene bank accession No.
B1	Borgou	PARAKOU (Guéma market)	BEN/30/2010	O	O/V*9	KC832983
B2			BEN/31/2010	O	O/V9	–
B3			BEN/32/2010	O	O/V8	KC832984
B4			BEN/33/2010	O	O/V10	KC832985
B5			BEN/37/2010	O	O/V1	–
B6			BEN/34/2010	O	O/V1	–
B14	Atacora	Toucountouna	BEN/40/2010	O	O/V11	KC832986
B15		Cobly	BEN/26/2010	O	O/V7	KC832981
B21	Alibori	Banikoara	BEN/35/2010	A	A/V5	KC832971
B23		Gogounou	BEN/27/2010	A	A/V1	–
B24			BEN/36/2010	A	A/V2	KC832972
B28			BEN/24/2010	A	A/V1	KC832970
B31			BEN/29/2010	A	A/V1	–
B32	Borgou	Kalalé	BEN/25/2010	O	O/V1	KC832980
B33			BEN/28/2010	O	O/V8	KC832982
B34			BEN/38/2010	O	O/V1	–
B35			BEN/41/2010	O	O/V1	–
B39		Sinendé (Guessou-Bani)	BEN/42/2010	O	O/V12	KC832987
B44		Bembéréké	BEN/19/2010	A	A/V6	KC832968
B48		Doubé N'dali	BEN/20/2010	O	O/V1	KC832977
B49		Bembéréké	BEN/21/2010	O	O/V5	KC832978
B51			BEN/22/2010	O	O/V6	KC832979
B52			BEN/14/2010	O	O/V4	KC832976
B53			BEN/15/2010	O	O/V4	–
B54			BEN/16/2010	O	O/V4	–
B55			BEN/17/2010	O	O/V4	–
B56		Nikki	BEN/23/2010	A	A/V4	KC832969
B58		Kalalé	BEN/18/2010	A	A/V3	KC832967
B59			BEN/43/2010	A	A/V3	–
B61		PARAKOU (Guéma market)	BEN/4/2010	O	O/V1	–
B62			BEN/5/2010	O	O/V1	–
B63			BEN/6/2010	O	O/V1	–
B64		PARAKOU Boko/Yacoumba	BEN/12/2010	O	O/V1	–
B65			BEN/10/2010	O	O/V1	–
B66			BEN/13/2010	O	O/V1	–
B67			BEN/7/2010	O	O/V1	–
B69			BEN/11/2010	O	O/V1	–
B70			BEN/8/2010	O	O/V1	–
B72		PARAKOU (Guéma market)	BEN/9/2010	O	O/V3	KC832975
B73			BEN/1/2010	O	O/V1	KC832973
B75			BEN/2/2010	O	O/V2	KC832974
B76			BEN/3/2010	O	O/V2	–

V*: genetic variant; sequences with 100% of homology in VP1 sequence are classified within the same variant.

SAT 1 and SAT 2 (Ayelet et al., 2009) were used to amplify VP1 sequence of FMDV from all samples found positive by rtRT-PCR pan-FMDV. The RT-PCR amplicons were sequenced on both strands. The sequences were assembled and verified using SeqMan software (DNAStar, Lasergene 8). The comparison and midpoint-rooted neighbor-joining trees of FMDV VP1 sequences from Benin with those from Africa available in the NCBI GenBank database were performed using the Clustal W method running within MEGA 5.05 software. The robustness of tree topology was assessed with 1000 bootstrap replicates by using the model in MEGA 5.05. Bootstrap values of >70 are shown at the relevant major nodes. Sequences showing 100% homology in VP1 were classified as one genetic variant. The complete VP1 nucleotide sequences generated in this study corresponding to each genetic variant but also collected from a specific geographic location were submitted to the NCBI GenBank database under the accession numbers indicated in Table 1. The midpoint-rooted neighbor-joining trees represent only prototypes of each genetic variant of FMDV isolates from Benin characterized in this study.

3. Results

3.1. FMDV detection and Serotype identification

Within 77 samples analyzed by rtRT-PCR pan-FMDV, only one sample (Alibori/Gogounou) was negative. FMDV was isolated from 42 samples. The rtRT-PCR negative sample was also VI negative.

The serotype of the 42 FMDV isolates was identified from infected cell cultures by Ag-ELISA and by sequencing of the VP1 coding sequence, amplified from RNA extracted both from the sample and from isolates. Thus, 33 isolates were identified as FMDV serotype O and 9 as FMDV

serotype A (Table 1). Serotype O was isolated from samples collected in Atacora department (2 isolates) and in Borgou department (31 isolates) (Table 1 and Fig. 1). Most of analyzed samples were collected from Parakou city animal's market (Guéma market). The serotype A was isolated from samples collected from Alibori department (5 isolates) and from Borgou department (4 isolates) (Table 1 and Fig. 1).

The serotype of the 34 samples rtRT-PCR (+)/VI(−), was analyzed from the sample suspension by VP1 amplification and sequencing. The expected fragments were amplified from 24 samples and sequencing revealed (type O = 13/ type A = 11). Despite positive result by rtRT-PCR pan-FMDV, the sequencing primers failed to amplify the VP1 region from 10 samples (data not shown).

3.2. Phylogenetic analysis: Serotype O

Type O viruses isolated from the same department in this study are closely related to each other (99.4–99.8%) (data not shown). However, the Borgou's and Atacora's isolates were less similar (91.2–91.4%) (data not shown) and it allows to classify them into two groups of isolates within the same topotype.

The VP1 sequences from Benin were compared to those available in the GenBank database (Fig. 2). The analysis revealed that the Benin isolates are mostly related to the FMDV from Ghana (O/Lam/GHA/2012) (93.5% and 93.6% of homology) and from Togo (O/TOG/4/2005) (93.1% and 91.6% of homology) classified within topotype West Africa. However, they show only 86.3% of homology with the

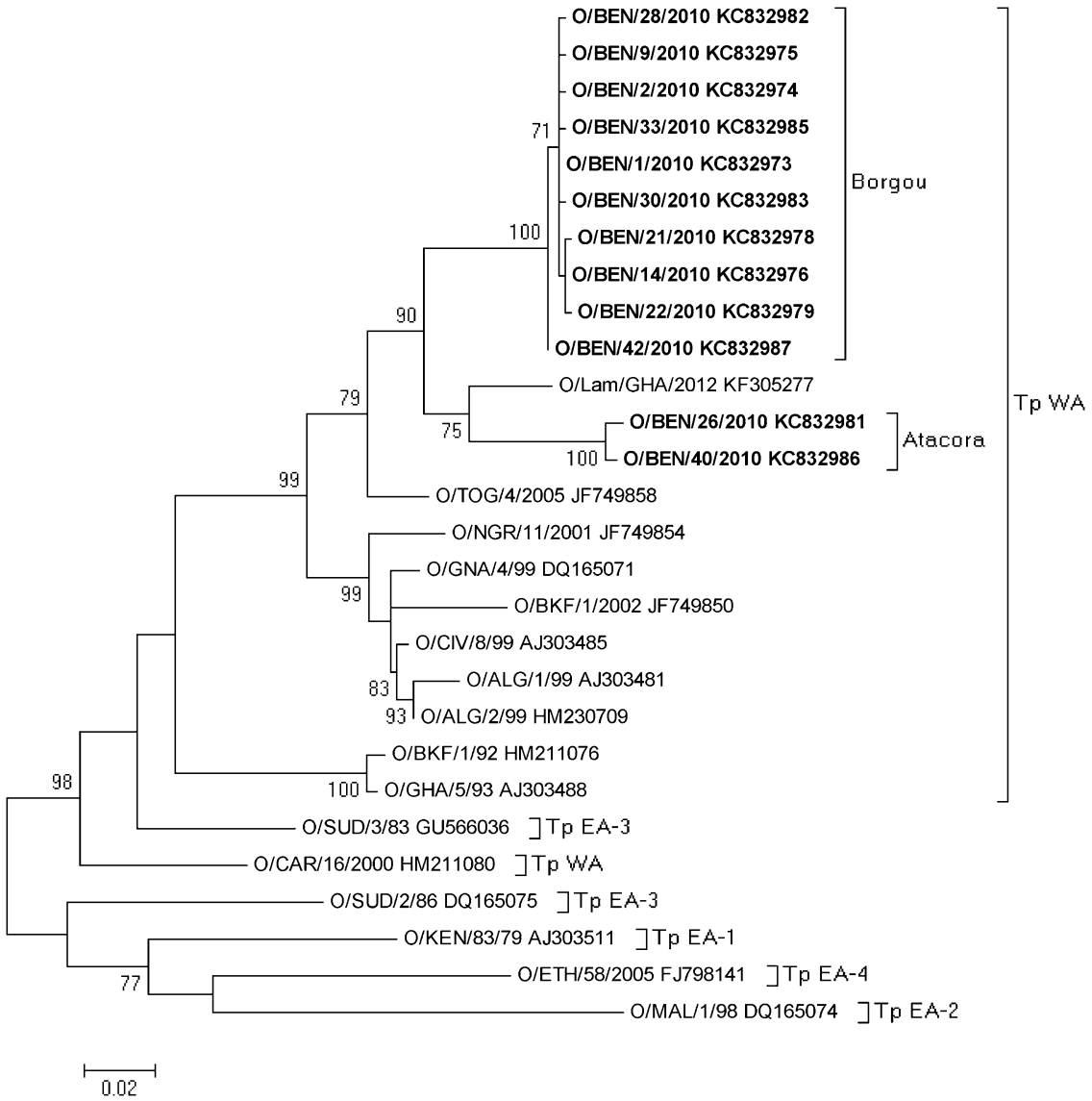

Fig. 2. Midpoint-rooted neighbor-joining tree showing the relationship between the VP1 sequences of serotype O isolated in Benin, the closest VP1 sequences of FMDV type O available in the GenBank database and prototype strains of East African topotypes 1–4. The percentage of taxa clustered together in a bootstrap test (1000 replicates) is shown next to the branches. The scale bar indicates nucleotide substitutions per site.

prototype strain of the West African topotype (O/GNA/5/93). The Benin strains are not closely related to the strains from East Africa (EA 1–4) (74.6–81.1% of similarity). However, they show fewer differences with strain O/SUD/83 from Sudan from EA-3 topotype (86.2% and 86.6% of similarity).

3.3. Phylogenetic analysis: serotype A

Viruses of serotype A were detected in Bourgou and Alibori. All VP1 sequences were very close (>98.0%) (data not shown). Thus, they may be classified into a single group.

The African strains of serotype A are classified into a unique topotype (Africa topotype) (Knowles and Samuel, 2003) and they are further subdivided in 8 different genotypes (GI-GVIII) (Knowles, 2009). Two Benin's VP1 sequences were compared with type A sequences available in the GenBank database (Fig. 3) and they are closely related to the strains of genotype VI (GVI) that occur in West Africa. They display the closest relationship with the strains: GAM/44/98 (92.0–92.2%), MAI/2/97 (91.7–92.2%), A/Senegal/2006 (91.1–91.3%) and CIV/4/95 (89.8–90.7%).

The phylogenetic analysis showed that the other African strains are not closely related to the Benin isolates and show a similarity between 82.8% (A/SUD/1/81, Africa topotype, genotype IV) and 74.8% (A/NIG/3/2009, Africa Topotype, genotype IV).

4. Discussion

The West African territories are considered as regions with continuous FMDV circulation. In a period of 2002–2009, the serotype O was characterized in: Burkina Faso, Togo, Niger, Nigeria, Cameroon, Senegal and Mali. The serotype A was confirmed in: Togo, Cameroon, Mali and Nigeria; and the serotype SAT 2 outbreaks were reported in: Cameroon, Niger and Nigeria. The SAT 1 serotype was last reported in 1981 (Nigeria) (FAO World Reference Laboratory for FMD, 2014).

In this study, 77 epithelial tissue samples collected between June and August 2010 from 3 different departments of Benin (Borgou, Atacora, and Alibori) were analyzed for detection and typing of FMDV.

The typing analysis of 42 isolates, by Ag-Capture Elisa and by sequencing, revealed 33 serotype O viruses and 9 serotype A viruses. Moreover, VP1 sequences amplified from samples with VI (−) coincide with corresponding FMDV isolates (same time and place) (data not shown). No evidence of co-circulation of two different serotypes

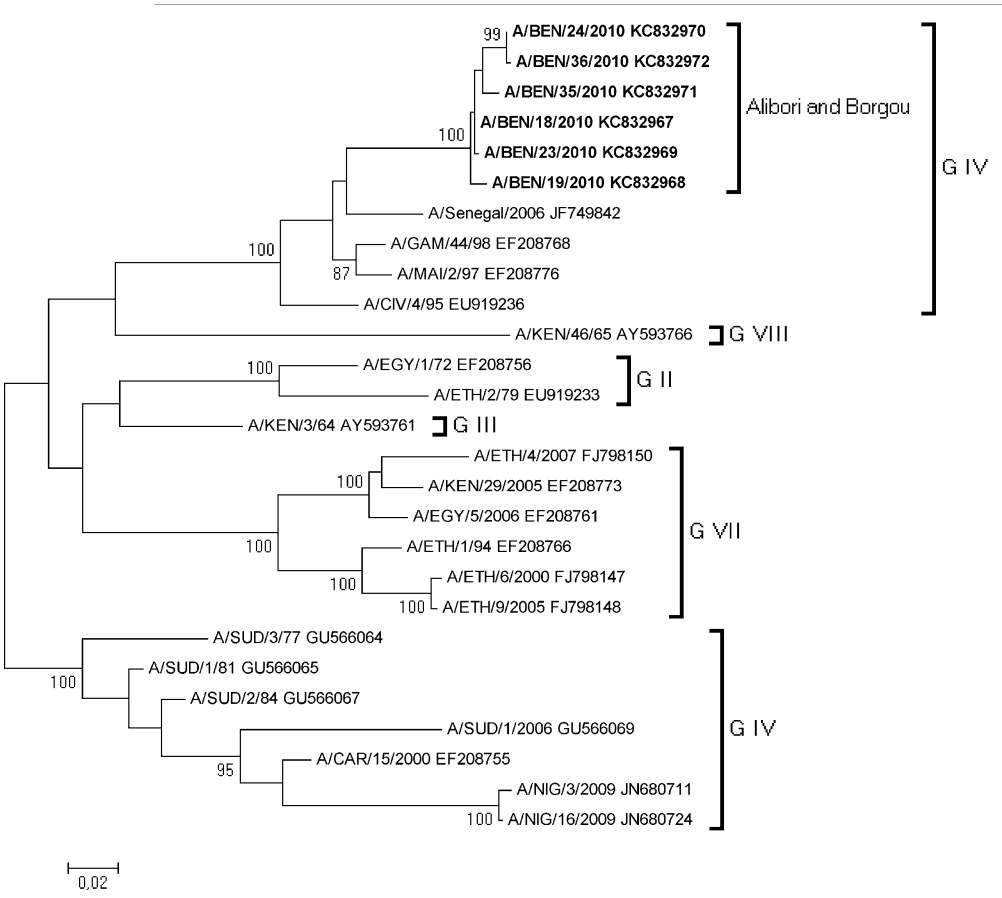

Fig. 3. Midpoint-rooted neighbor-joining tree showing the relationship between the VP1 sequences of serotype A strains isolated in Benin and the closest VP1 sequences of FMDV type A from West and East Africa available in the GenBank database and the FMDV reference topotypes A. The percentage of taxa clustered together in a bootstrap test (1000 replicates) is shown next to the branches. The scale bar indicates nucleotide substitutions per site.

within the same animal was found since only one serotype was amplified from each sample. It was not possible to amplify the VP1 sequence from 10 samples found positive by pan-FMDV rtRT-PCR. This may be due to several factors such as the insufficient quality of the samples (degradation of the genome), lower analytical sensitivity of the sequencing VP1 RT-PCRs or primer mismatches.

Sequence coding for the FMDV VP1 protein has been used to investigate the relationship between different isolates of the virus (Beck and Strohmaier, 1987). It is assumed that, viruses with <5% of nucleotide differences in the VP1 sequence are considered as closely related and those with >15% as unrelated (Knowles and Samuel, 2003). Thus, the analysis of Benin's VP1 sequences revealed two different groups of FMD type O viruses (8.6–8.8% of difference): one in the Atacora department and the second in the Borgou department. As the level of difference between them is not >15%, we conclude that they are related but probably, came from two different sources. The type O viruses were detected in almost all communes from Borgou. Although, we detected type O virus near the border with the Alibori department, we did not find evidence of type O in this department. But number of samples from Alibori was not sufficient to definitively conclude on the absence of this serotype. Benin's serotype A viruses are very close (VP1 < 1.4%) and were recovered from samples collected in Alibori and Borgou but not in Atacora. The type A was isolated from Borgou's communes that are neighboured with Alibori. Thus, this outbreak may result from animal movements between these departments (Dehoux and Hounsou-Ve, 1991; Couacy-Hymann et al., 2006).

VP1 sequence analysis of the Benin isolates with other sequence data available in the GenBank database indicated that these viruses are related to strains previously isolated in West Africa. The Benin isolates of serotype O belong to the West Africa (WA) topotype (Fig. 2). The Benin isolates of serotype A belong to the topotype Africa of GVI (Fig. 3). Unfortunately, these data do not allow a clear identification of the source of introduction of FMDV. The knowledge about recent strains circulating in West Africa, in particular from the neighboring countries of Benin would be of a great value to answer to this question.

The epidemiological studies of FMDV are difficult to carry out in this region of Africa due to uncontrolled animal movements with poor administrative surveillance and porous borders. Furthermore, problems in collecting and transporting suspected samples to laboratories make the virus characterization difficult (Vosloo et al., 2002). The recent observations on FMD incidence in West Africa have identified certain regions as constant reservoirs of FMDV. Two of them are situated on the border areas of Benin (Couacy-Hymann et al., 2006). In most of the West and Central African countries the cross-border transhumance and animal trade are frequent (Kamuanga et al., 2008; Guibert et al., 2009). Therefore, FMDV may be introduced to Benin from infected zones through these animal movements. In parallel, the constant "endemic situation" of FMD in Benin, with the same lineages that persists and have caused the outbreaks in this country since many years should be considered.

This study, for the first time to our knowledge, described the isolation and characterization of FMDV strains circulating in Benin. This information should be confronted with the recent data from neighboring countries to build a regional strategy to control the spread of FMD in West Africa. Also, the regular sampling campaigns, with the monitoring of inter-regional and trans-boundary animal movements, are needed to keep track of the virus's spread and evolution. The Benin's isolates may be useful for vaccine matching studies in view of initiating effective vaccination campaigns in this region. Additional studies of the full genome sequencing of the representative isolates could contribute to a better understanding of the virus and shed light on other changes leading to the emergence of new strain variants that could hinder vaccine protection. Epidemiological and molecular information on FMDV combined with increased awareness in the African population of the seriousness of the FMD problem may lead to successfully controlling the spread of this major animal disease in Africa.

Acknowledgments

Houndje Evelyne is a PhD student of the University of Liege (UREAR-ULg) supported by the Belgian Development Collaboration Department through the Institute of Tropical Medicine (Antwerp) and ANSES. We thank Aplogan Luc, Yokossi Tcharo and animal health technicians who have participated to this work.

References

Ayelet, G., Mahapatra, M., Gelaye, E., Egziabher, B.G., Rufeal, T., Sahle, M., Ferris, N.P., Wadsworth, J., Hutchings, G.H., Knowles, N.J., 2009. Genetic characterization of foot-and-mouth disease viruses, Ethiopia, 1981–2007. Emerg. Infect. Dis. 15 (September (9)), 1409–1417.

Bastos, A.D.S., Haydon, D.T., Sangaré, O., Boshoff, C.I., Edrich, J.L., Thomson, G.R., 2003. The implications of virus diversity within the SAT 2 serotype for control of foot-and-mouth disease in sub-Saharan Africa. J. Gen. Virol. 84 (June (Pt 6)), 1595–1606.

Beck, E., Strohmaier, K., 1987. Subtyping of European foot-and-mouth disease virus strains by nucleotide sequence determination. J. Virol. 61 (May (5)), 1621–1629.

Brehm, K.E., Ferris, N.P., Lenk, M., Riebe, R., Haas, B., 2009. Highly sensitive fetal goat tongue cell line for detection and isolation of foot-and-mouth disease virus. J. Clin. Microbiol. 47 (October (10)), 3156–3160.

Callahan, J.D., Brown, F., Osorio, F.A., Sur, J.H., Kramer, E., Long, G.W., Lubroth, J., Ellis, S.J., Shoulars, K.S., Gaffney, K.L., Rock, D.L., Nelson, W.M., 2002. Use of a portable real-time reverse transcriptase-polymerase chain reaction assay for rapid detection of foot-and-mouth disease virus. YAVMA 220 (11), 1636–1642.

Couacy-Hymann, E., Aplogan, G.L., Sangaré, O., Compaoré, Z., Karimu, J., Awoueme, K.A., Seini, A., Martin, V., Valarcher, J.F., 2006. Etude rétrospective de la fièvre aphteuse en Afrique de l'Ouest de 1970 à 2003. Rev. Sci. Tech. Off. Int. Epiz. 25 (3), 1013–1024.

Dehoux, J.P., Hounsou-Ve, G., 1991. Epizoootie de fièvre aphteuse au Nord-Bénin Durant la saison sèche 1990–1991. Revue Elev. Méd. Vét. Pays trop. 44 (3), 261–262.

FAO, 2014. World Reference Laboratory for FMD. Molecular Epidemiology Reports. Available at: http://www.wrlfmd.org/fmd_genotyping/index.html (accessed 05.02.14).

Ferris, N.P., Dawson, M., 1988. Routine application of enzyme-linked immunosorbent assay in comparison with complement fixation for diagnosis of foot-and-mouth and swine vesicular diseases. Vet. Microbiol. 16, 201–209.

Grubman, M.J., Baxt, B., 2004. Foot-and-mouth disease. Clin. Microbiol. Rev. 17 (April (2)), 465–493.

Guibert, B., Banzhaf, M., Soulé, G., Balami, H., Idé, G., 2009. Etude régionale sur les contextes de la commercialisation du bétail/accès aux marches

et défis d'amelioration des conditions de vie des communautés pastorals. IRAM study. Available at: http://www.iram-fr.org/documents/Rapport%20iram%20commercialisation%20betail%20def.pdf (accessed 13.09.13).

Kamuanga, M.J.B., Somda, J., Sanon, Y., Kagone, H., 2008. Livestock and regional market in the Sahel and West Africa: potential and challenges: Study carried out within ECOWAS Commission and SWAC/OECD partnership on the future of livestock in the Sahel and West Africa, Sahel and West Africa Club/OECD, Issy-les-Moulineaux, France, pp. 150.

Knowles, N.J., 2009. FMD distribution in Africa: types, topotypes and virus pools and their relevance for ecosystem based surveillance and control programs. Oral presentation at the OIE/FAO GF-TADs for Africa and ALive Platform Frameworks.In: Workshop on the Development of Action Plans for Improved Surveillance and Control of FMD in Africa, Nairobi, Kenya, 26–30 January 2009.

Knowles, N.J., Samuel, A.R., 2003. Molecular epidemiology of foot-and-mouth disease virus. Virus Res. 91 (January (1)), 65–80.

OIE, 2012. Manual of Diagnostic Tests and Vaccines for Terrestial Animals, 7th ed. (Chapter 2.1.5).

Sangare, O., Bastos, A.D.S., Marquardt, O., Venter, E.H., Vosloo, W., Thomson, G.R., 2001. Molecular epidemiology of serotype O foot-and-mouth disease virus with emphasis on West and South Africa. Virus Genes. 22 (June (3)), 345–351.

Sangare, O., Bastos, A.D.S., Venter, E.H., Vosloo, W., 2003. Retrospective genetic analysis of SAT-1 type foot-and-mouth disease outbreaks in West Africa (1975–1981). Vet Microbiol. 93 (June (4)), 279–289.

Thompson, D., Muriel, P., Russel, D., Osborne, P., Bromley, A., Rowland, M., Creigh-Tyte, S., Brown, C., 2002. Economic costs of foot and mouth disease outbreak in United Kingdom in 2001. Rev. Sci tech. Off. Int. Epiz. 21 (3), 675–687.

Toussaint, J.F., Sailleau, C., Bréard, E., Zientara, S., De Clercq, K., 2007. Bluetongue virus detection by two real-time RT-qPCR targeting two different genomic segments. J. Virol. Methods 140, 115–128.

Vosloo, W., Bastos, A.D.S., Sangare, O., Hargreaves, S.K., Thomson, G.R., 2002. Review of the status and control of foot and mouth disease in sub-Saharan Africa. Rev. sci. tech Off. int. Epiz. 21 (3), 437–449.