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Short Communication

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

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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

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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.

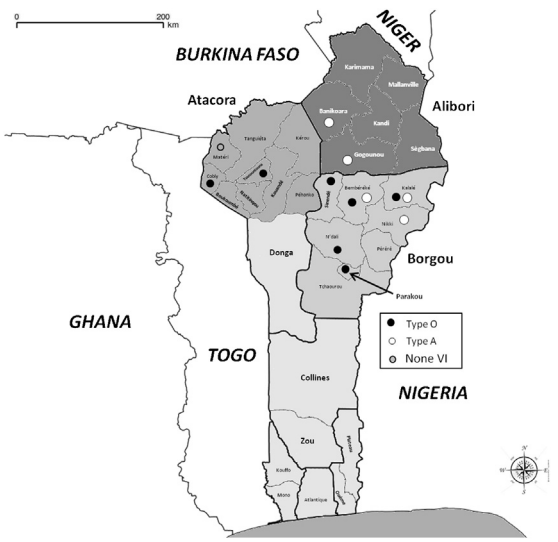

Fig. 1. Map of Benin showing the places of sample collection and the geographical location of FMDV serotypes isolated in this study. NONE VI #: None FMDV was isolated from the samples collected in that place.

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,