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Phylogenetic analysis of foot-and-mouth disease virus type O re-emerging in free areas of South America

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Abstract

The nucleotide sequences of the complete VP_1-coding region of foot-and-mouth disease viruses (FMDV), type O, isolated during the recent emergencies of the disease in free areas of South America (Mato Grosso do Sul, Brazil, October 2005, and Corrientes, Argentina, February 2006), were determined. Also established were the complete VP_1-coding sequences of viruses occurring in neighbouring locations between the years 2000 and 2003. A phylogenetic analysis was performed based on comparison with continental relevant field and vaccine strains, as well as with extra-continental representative viruses.

The results show that the emergencies in Argentina and Brazil were caused by viruses presenting 93% genetic relatedness. Both variants are endogenous to South America, as they were placed within the Europe–South America topotype. When compared with the continental viruses available for the phylogenetic studies, they show the closest relationship with viruses responsible for previous emergencies in neighbouring free areas, or for sporadic outbreaks in the adjacent places with advanced eradication stages, presenting similarity values of at least 90% among them, and clustering together in a unique lineage. This lineage represents the only one sporadically appearing in the Southern Cone and differs from those including viruses presently circulating in the Andean region, reflecting the different livestock circuits and epidemiological scenarios.

Keywords: Foot-and-mouth disease virus; Molecular epidemiology; Sequence analysis; Europe–South America FMDV topotype

1. Introduction

Foot-and-mouth disease virus (FMDV) is a member of the *Picornaviridae* family that causes a highly contagious vesicular disease of cattle and other cloven-hoofed animals (Bachrach, 1968; Pereira, 1981). Although mortality due to the disease is very low and mostly restricted to young animals, drastic decrease in productivity and working capacity of the animals causes great losses to the livestock industry. The disease has an important socio-economic impact in countries where it is endemic (Astudillo et al., 1990; Perry et al., 1999), provokes huge economic consequences when outbreaks occur in disease free regions (emergencies) (Correa Melo et al., 2002; Huang et al., 2000), and is considered one of the most important barriers to world trade of livestock and animal products.

The virus possesses a single-stranded positive RNA molecule of about 8200 nucleotides, within an icosahedral capsid made of 60 copies each of four proteins VP_1, VP_2, VP_3, and VP_4

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(Racaniello, 2001). As recorded for many other RNA viruses, FMDV is highly variable (Domingo et al., 1990; Holland et al., 1982, 1992). The viruses can be differentiated into seven serotypes (O, A, C, SAT-1, SAT-2, SAT-3 and Asia-1); of which, only types O, A and C are present in South America.

It has been shown that VP_1 is the most variable among the capsid polypeptides and is considered to be the major immunogenic protein, since it contains a linear antigenic site able to induce neutralizing antibodies sufficient to protect animals against the disease (Bittle et al., 1982; DiMarchi et al., 1986). Nucleotide sequencing of the complete or partial genomic region coding for this protein has been extensively used for molecular epidemiology studies on FMD. Since the first analysis made by Beck and Strohmaier (1987), an important amount of information has been gathered (Ansell et al., 1994; Bergmann et al., 1992; Carrillo et al., 1990; Malirat et al., 1994; Piccone et al., 1988; Saiz et al., 1993; Vosloo et al., 1992, review in Knowles and Samuel, 2003) so that it is now possible to study the relationship between strains of FMDV, and to group the different isolates according to their genetic relationships into phylogenetic trees. Recent data have demonstrated the usefulness of such phylogenetic studies in establishing the epidemiological links among viral isolates, following geographical movement of strains and facilitating identification of the source of virus strains (Araujo et al., 2002; Bastos et al., 2001; Bergmann et al., 2005b; Hemadri et al., 2000; Huang et al., 2001; Knowles et al., 2005; König et al., 2001; Samuel and Knowles, 2001). This kind of study becomes particularly important to trace the origin of outbreaks in already free regions, with or without vaccination, or regions with advanced eradication plans, where the knowledge of the putative origin of viruses emerging or re-emerging is necessary not only for control measures but also to give confidence to the sanitary plans (Bergmann et al., 2005b; Knowles et al., 2001).

In the Southern Cone of South America, the status of free of FMD by the International Organization of Animal Health (OIE) was obtained in the late 1990s as a result of a strong eradication program initiated in 1988 in the Continent. During the 1990s, the following countries were recognized by the OIE as FMD-free where vaccination is practiced: Uruguay, 1994, Argentina, 1997, Paraguay, 1997. In 1998 a zone comprising the southern states of Brazil acquired the status of FMD-free zone where vaccination is practiced, and in 2000, the state of Mato Grosso do Sul also gained this status. Uruguay stopped vaccination in 1994 and became free without vaccination in 1996. Argentina and Paraguay discontinued vaccination in 1999 and Argentina was recognized free without vaccination in 2000 (Bergmann et al., 2005a).

Later in the year 2000, some outbreaks of serotype O put this region in an emergency status. Brazil (Rio Grande do Sul state), Argentina (Corrientes and Misiones provinces) and Uruguay (Artigas department) had sporadic episodes between July and October (Correa Melo et al., 2002). Afterwards, type O virus re-emerged in Paraguay (Canindeyú, 2002 and Pozo Hondo, 2003), and also appeared in Bolivia (Chuquisaca and Potosí, 2003).

Recently, 33 FMD outbreaks were registered in Mato Grosso do Sul, Brazil, during October–December, 2005, and one outbreak in Corrientes, Argentina, February, 2006, in which virus isolation and typing, recorded FMDV type O. This paper reports the application of nucleotide sequencing to perform a phylogenetic study of FMD type O viruses from these outbreaks. Also determined and included in the analysis were the complete VP_1-coding sequences of viruses responsible for previous outbreaks in FMD-free regions, or sporadic episodes in areas with advanced eradication programs, registered in neighbouring areas, between the years 2000 and 2003. The sequences were compared with selected South American field and vaccine strains, as well as with exogenous viruses.

The re-emergence of FMDV type O had a number of interesting features, which are discussed together with their implications for the prospects of re-introduction of FMD in this region.

2. Materials and methods

2.1. Viruses and cells

The designation and origin of viruses examined in this study together with FMDV sequences available from gene bank and used in this analysis are listed in Table 1. The geographic location of FMD Type O outbreaks recorded in the southern cone of South America between 1998 and 2006, included in this study, is depicted in Fig. 1.

Most viral RNAs were directly extracted from epithelium samples, although, for one of them, particularly the one recovered from oesophageal–pharyngeal fluid, a passage in BHK-21 cell cultures was needed to increase the RNA mass necessary for the studies.

2.2. Oligonucleotide primers

Two oligonucleotide primers already described (Malirat and Bergmann, 2003) were used in this study to amplify and sequence the complete VP_1-coding region, rendering amplification fragments of 789 bp. Oligonucleotide sequences are, for the forward primer: 5′-AATTACACATGGCAAGGCCGACGG-3′; and for the reverse one: 5′-GAAGGGCCCAGGGTTGGACTC-3′.

Fig. 1. Geographic location of FMD outbreaks analyzed in this study. The stars indicate the locations of the referred outbreaks type O recorded in the southern cone of South America between the years 1998 and 2006, included in this study.

Table 1
Designation and origin of foot-and-mouth disease type O viruses studied

Virus designation	Geographical location	Date collected	Genebank accession number	References
O/MS/Bra/98	Naviraí, Mato Grosso do Sul, Brazil	1998	DQ834704	This work
O/Argentina/00	Argentina	2000	DQ834705	This work
O/RS/Bra/00	Jóia, Rio Grande do Sul, Brazil	23 August 2000	DQ834706	This work
O/Artigas/Uru/00	Artigas, Uruguay	23 October 2000	DQ834707	This work
O/Tarija/Bol/00	Chaco, Tarija, Bolivia	21 November 2000	DQ834708	This work
O/Tarija/Bol/01	Moto Méndez, Tarija, Bolivia	28 July 2001	DQ834709	This work
O/Canindeyú/Par/02[a]	Corpus Christi, Canindeyú, Paraguay	16 October 2002	DQ834710	This work
O/Pozo Hondo/Par/03	Pozo Hondo, Boquerón, Paraguay	7 July 2003	DQ834711	This work
O/Chuquisaca/Bol/03	Siles, Chuquisaca, Bolivia	7 July 2003	DQ834712	This work
O/Potosí/Bol/03	Cornélio Saavedra, Potosí, Bolivia	13 July 2003	DQ834713	This work
O/MS(1a)/Bra/05	Eldorado (farm 1), Mato Grosso do Sul, Brazil	01 October 2005	DQ834714	This work
O/MS(1b)/Bra/05	Eldorado (farm 1), Mato Grosso do Sul, Brazil	01 October 2005	DQ834715	This work
O/MS(1c)/Bra/05	Eldorado (farm 1), Mato Grosso do Sul, Brazil	01 October 2005	DQ834716	This work
O/MS(1d)/Bra/05	Eldorado (farm 1), Mato Grosso do Sul, Brazil	07 October 2005	DQ834717	This work
O/MS(1e)/Bra/05	Eldorado (farm 1), Mato Grosso do Sul, Brazil	07 October 2005	DQ834718	This work
O/MS(1f)/Bra/05	Eldorado (farm 1), Mato Grosso do Sul, Brazil	07 October 2005	DQ834719	This work
O/MS(1g)/Bra/05	Eldorado (farm 1), Mato Grosso do Sul, Brazil	07 October 2005	DQ834720	This work
O/MS(1h)/Bra/05	Eldorado (farm 1), Mato Grosso do Sul, Brazil	07 October 2005	DQ834721	This work
O/MS(1i)/Bra/05	Eldorado (farm 1), Mato Grosso do Sul, Brazil	07 October 2005	DQ834722	This work
O/MS(1j)/Bra/05	Eldorado (farm 1), Mato Grosso do Sul, Brazil	07 October 2005	DQ834723	This work
O/MS(2)/Bra/05	Japorã (farm 2), Mato Grosso do Sul, Brazil	08 October 2005	DQ834724	This work
O/MS(3)/Bra/05	Eldorado (farm 3), Mato Grosso do Sul, Brazil	08 October 2005	DQ834725	This work
O/MS(4)/Bra/05	Japorã (farm 4), Mato Grosso do Sul, Brazil	08 October 2005	DQ834726	This work
O/Corrientes/Arg/06	San Luis del Palmar, Corrientes, Argentina	02 May 2006	DQ834727	This work
O/Irigoyen/Arg/83	H. Irigoyen, Buenos Aires, Argentina	September 1983	DQ789075	Malirat et al. (1994)
O/Luján/Arg/83	Lujan, Buenos Aires, Argentina	1983	J308705	König et al. (2001)
O/Irigoyen/Arg/82	Yrigoyen, Argentina	1982	Z21862	Saiz et al. (1993)
O1/Caseros/Arg/67	Caseros, Santa Fé, Argentina	February 1967	U82271	Tami et al. (1997)
O1/Campos/Bra/58	Campos, São Paulo, Brazil	1958	K01201	Cheung et al. (1983)
O1/Kaufbeuren/FRG/66	Kaufbeuren, Germany	1966	X00871	Forss et al. (1984)
O1/BFS I UK/67	United Kingdom	1967	J02185	Makoff et al. (1982)
O/ALG/1/99	Souidania, Govrn. of Greater Algiers, Algeria	February 1999	AJ303481	Samuel and Knowles (2001)
O/UGA/5/96	Mbarara, Uganda	17 January 1996	AJ296327	Samuel and Knowles (2001)
O/TAW/81/97	I-lan, Taiwan, POC	24 April 1997	AJ296321	Samuel and Knowles (2001)
O/TAI/4/99	Mae Hong Son, Thailand	01 March 1999	AJ303536	Samuel and Knowles (2001)
O/VIT/7/97	Har, Yrongpa, Gialai, Vietnam	31 August 1998	AJ296328	Samuel and Knowles (2001)
O/UKG/3/01	Brentwood, Essex, Great Britain	February 2001	AJ311720	Knowles et al. (2001)
O/UKG/12/0	1Brentwood, Essex, United Kingdom	February 2001	AJ311724	Knowles et al. (2001)
O1/Manisa/Tur/69	Manisa, Turkey01/04/1969		AJ251477	Aktas and Samuel (2000)

[a] Collected from oesophageal pharyngeal fluid; 1 passage in BHK-21 cells was necessary previous to RT-PCR amplification.

2.3. Viral RNA extraction and reverse transcription-polymerase chain reaction (RT-PCR)

The general protocol was as previously described (Malirat and Bergmann, 2003). Total RNA was extracted from epithelium samples or from infected cell culture supernatants using Trizol reagent (Invitrogen) according to the manufacturer's protocol.

Reverse transcription of the viral RNAs (5 μl, 3–5 μg RNA) was carried out using 50 ng of random primers and 50 units of Superscript II reverse transcriptase (Invitrogen) and incubating at 42 °C for 60 min, followed by extension at 70 °C, 15 min, in a 25 μl reaction mix containing 20 mM Tris–HCl (pH 8.4), 50 mM KCl, 2.5 mM $MgCl_2$, 10 mM dithyothreitol and 0.6 mM of each dNTPs.

In vitro amplification was carried out with a programmable thermocycler GeneAmp PCR system 9700 (Applied Biosystems). Each reaction was performed in a final volume of 50 μl of a reaction mixture containing: 5 μl cDNA, 0.5 μM of each primer, 2.5 U of *Thermus aquaticus* polymerase (Invitrogen), 0.2 mM each dNTP (dATP, dCTP, dGTP, dTTP) and 1.5 mM $MgCl_2$ in 20 mM Tris–HCl (pH 8.4), 50 mM KCl, 0.1% Triton X-100. After denaturing 5 min at 94 °C, reaction mixtures were incubated at 94 °C for 1 min, followed by cooling to, and then holding at 60 °C for 45 s, to allow primer annealing. Each cycle ended with a chain-elongation step at 72 °C for 2 min. This process was repeated 30 times. After the last cycle, polymerization was continued at 72 °C for 5 min.

The amplified product was purified by band excision from 1% agarose gel electrophoresis, followed by chromatography in affinity columns (Promega) and the recovered material was quantified by band intensity comparison with DNA mass and molecular weight marker (Invitrogen) in 1% agarose gel electrophoresis.

2.4. Nucleotide sequence determination and analysis

The nucleotide sequences were determined from 20 to 60 ng of the purified amplicon, using the Big Dye Terminator kit 3.1 (Applied Biosystems), according to the manufacturer's procedure, in a thermocycler, performing 40 cycles of 94 °C, 45 s; 50 °C, 30 s; 60 °C, 4 min. After cycles, the reaction product was purified by exclusion chromatography (CentriSep columns, Princeton separations); the recovered material was dyed. For reading, the dyed samples were ressuspended in formamide 10%, as recommended for use in an ABI Prism 3100 Avant Genetic Analyzer sequencing machine. The sequences determined in this study have been submitted to the EMBL/GeneBank/DDBJ databases; accession numbers are shown in Table 1.

Chromatograms obtained for each individual reaction were edited manually to avoid misreading of peak dyes. Nucleotide sequences were analyzed on an IBM compatible personal computer using for editing and alignment the program BioEdit, version 5.0.2.1. All pairwise comparisons were performed by giving each base substitution equal statistical weight. An unrooted tree was constructed according to sequence relatedness across the interval of nucleotides of the VP_1 gene (1-633 n, covering the ultimately 211 amino acids recognized for VP_1, as referred in Knowles et al., 2005, using the neighbor-joining method as implemented in the computer program MEGA, version 3.1 (Kumar et al., 2004). Bootstrap resampling analysis was performed with 1000 replicates, as implemented in the program.

3. Results

3.1. Phylogenetic analysis of type O emergency viruses in South America

The complete nucleotide sequence of the VP_1-coding region was determined for 14 type O viruses obtained from epithelium samples collected in four of the affected farms, in the counties of Eldorado and Japorã, Mato Grosso do Sul, Brazil, and in the farm affected in Corrientes, Argentina. Also sequenced was the complete VP_1 coding region of viruses responsible for previous emergencies or sporadic episodes registered in the neighbouring areas between the years 2000 and 2003 (Table 1 and Fig. 1). These sequences were aligned and compared with those of South American representative type O viruses, including strains used for vaccine formulation. Representative strains of topotypes exogenous to South America were also included. A phylogenetic tree generated using the neighbor-joining method is shown in Fig. 2.

All viruses studied from the outbreaks in Mato Grosso do Sul showed a limited degree of variation in the VP_1 gene, with values of over 99.3% genetic relatedness among them, and with only three amino acid changes observed. Isolates collected from the same farm were almost identical, so the within-herd distances ranged from 0% to 0.6%. The same values were obtained when comparing isolates from different herds, sampled within a few days. The virus isolated in Corrientes, Argentina, 4 months later,

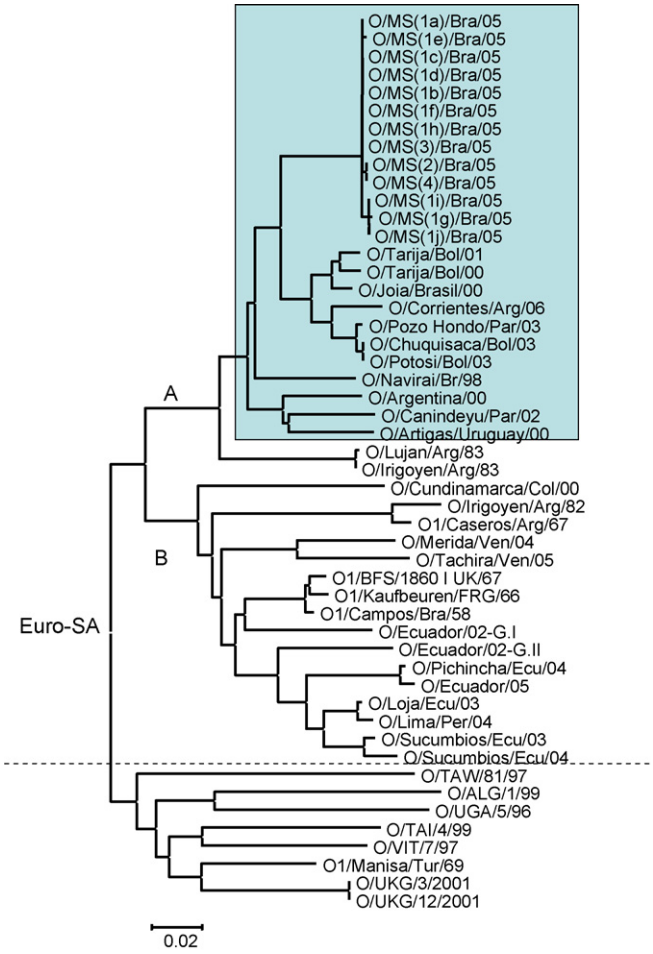

Fig. 2. Phylogenetic tree showing the genetic relationships of FMD virus type O isolates in South America. Also included were representative strains of the other O topotypes. The p-distances were calculated based on the comparison of the 633 nucleotides of the VP_1 gene. The tree was constructed using the Mega 3.1 program. Viruses in the Southern Cone lineage are highlighted inside the square. Accession numbers are listed in Table 1, except for viruses from the Andean region (obtained from the PANAFTOSA genetic bank, manuscript in preparation).

was not very closely related to the strain recovered in Mato Grosso do Sul, Brazil, and presented genetic relatedness of about 93%, and differences in 10–13 of the 211 amino acids in the VP_1 protein.

These isolates were also compared to the viruses responsible for the previous emergencies or sporadic cases in the southern cone since the year 2000, as well as with other relevant strains from the continent, and with representative exogenous viruses. From the results presented in Fig. 2, it becomes evident that all type O viruses sequenced from South America belong to the Europe–South America topotype. Viruses in this topotype occurred in various lineages and, based on the phylogenetic analysis, it can be concluded that all isolates recovered from the southern cone between 2000 and 2006 fall within a unique lineage, and showed percentages of nucleotide sequence identity of at least 89% among them, and of 93% mean within the group. This lineage also included the O/Naviraí/MS/Bra/98 virus, which represented the last isolate in the State of Mato

Grosso do Sul, prior to its recognition as free with vaccination by OIE.

When compared with other strains in the databank, particularly with those circulating in the past three decades, they show the closest relatedness with viruses O/Irigoyen/Arg/83 and O/Luján/Arg/83, with which they have approximately 87–89% sequence identity.

When compared with the strain used for vaccine preparation (O1/Campos/Bra/58), all the emergency/sporadic viruses recorded values of 15–16% nucleotide sequence difference and were placed in a different group.

The phylogenetic analysis also included comparison with circulating representative isolates occurring in the last 6 years in the Andean region. The results clearly show that the emergency viruses recorded in the southern cone between the years 2000 and 2006 are all clustered together in a different lineage than those grouping the presently circulating Andean strains.

A careful analysis of the Europe–South America topotype, allowed identifying two main groups. Strains in group A, in which O isolates of the mentioned emergencies are placed, showed a similarity of over 87% among them. Within this group, five different branches were observed. One of these, carries the older viruses available for the group (O/Irigoyen/Arg/83 and O/Luján/Arg/83), which were included in this analysis because of their historical value. The remaining four branches show mean distances of 7–9.5% among them and included all the southern cone variants from the emergencies, which, as mentioned above, belong to a unique lineage. These four branches can be described as follows: one of them in which a single isolate, O Navirai/Bra/98 is included. Another branch grouped together viruses appearing in 2000, 2001, 2003, and 2006 in four different countries, Argentina, Bolivia, Brazil, and Paraguay (O/Joia/Bra/00, O/Tarija/Bol/00, O/Tarija/Bol/01, O/Potosí/Bol/01, O/Pozo Hondo/Par/03, O/Chuquisaca/Bol/03, O/Potosi/Bol/03, and O/Corrientes/Arg/06). These isolates are closely related, with values of at least 95.2% similarity among them, and could be considered as the same strain. Another branch carried only the variant responsible for the reappearance in Mato Grosso do Sul, Brazil (13 isolates). The fourth branch included two viruses from the emergencies in Argentina and Uruguay in the year 2000 together with the virus recovered from oesophageal–pharyngeal fluid from one animal in the department of Canindeyú, Paraguay in 2002, the three of them diverging in 6.5–7.0%.

The other group (B), in which the vaccine strain O1 Campos/Bra/58 was placed, showed nucleotide sequence identity values of over 83% among the isolates, and included four lineages, three of them bearing the strains circulating since 2000 in the Andean region. The genetic divergence among the Andean lineages was of 13–15% (manuscript in preparation; Fig. 2).

Between the two groups (A and B) divergence values ranged from 15% to 20%, with a mean average of 17%. None of the South American isolates was related to the pandemic PanAsia strain, responsible for O outbreaks in Middle East Asia and registered in 2001 in the UK and the European continent (Fig. 1).

4. Discussion

The re-introduction of FMD into free areas of the southern cone of South America had a great impact for governments, livestock production and market sector, and the public as a whole. In this regard, the investigation of the molecular epidemiology of FMDV strains re-appearing in this region was of utmost importance to provide further insights into the epidemiology of the disease and to assist in the planning of control strategies (Correa Melo et al., 2002).

The present study focused on the type O viruses causing the emergencies or sporadic episodes in this region. Phylogenetic analysis showed that all emergency/sporadic viruses in the southern cone circulating between the years 2000 and 2006 clustered as part of a clade, supported by 97% bootstrap value. The clustering profile showed considerable genetic relationships between viruses in countries with common boundaries.

When compared with the available sequences from the viruses that circulated in the continent in the past two decades, the southern cone emergency lineage showed a closer relationship with viruses O/Irigoyen/Arg/83 and O/Luján/Arg/83, with relatedness values of ca. 87–89%, being somehow apart from strains recorded in the same countries, and historically referred as of national importance, like O/Irigoyen/Arg/82 and O/Caseros/Arg/67 (Fig. 1) and O1/Brazil/80, O1/Brazil/70, and O/Argentina/77 (Bergmann et al., 2005b).

In terms of phylogenetic interpretations, FMDV that differ in 2–5% from each other are generally believed to originate from the same epizootic (Samuel et al., 1997). In this context this analysis indicates that the viruses from the last emergencies recorded in Mato Grosso do Sul, Brazil (October 2005) and Corrientes, Argentina (February 2006) within a 4-month period, are distant enough (8% divergence) to define that they are not directly linked.

A major problem when discussing the relationship between isolates and their sequence data, is deciding what the observed genetic distances actually mean. In general, distances <5% are considered to indicate very closely related strains (Samuel, 1997). In contrast, distances of more than 15% have been considered sufficient to classify isolates as belonging to lineages that evolved independently (Samuel and Knowles, 2001). This leaves a range of 5–15% phylogenetic distances that are undefined.

Despite having been sampled over a period of about 7 years, the isolates of the emergencies or sporadic episodes of the southern cone, between the years 2000 and 2006 show a group mean divergence of 7%, being placed in a unique lineage. This genetic relationship found for the isolates from these five countries suggests that, besides being endogenous, trans-border movement is a major cause for disease dissemination. One could speculate that the sharing of clades between countries could be largely influenced by the socio-economic situation in that area at a given point in time.

Differences in the genetic sequences of viruses from the same serotype do not necessarily reflect differences in antigenicity (Esterhuysen, 1994). Conversely, it has also been shown that very limited genetic variations in the immunodominant region

can alter the antigenic specificity of FMDV isolates (Mateu et al., 1990, 1996). In the isolates included in this study, in spite of the genetic differences found between type O emergency viruses recorded between the years 2000 and 2006 and the strain used for vaccine production (O1/Campos/Bra/58), effective protection against these field viruses was achieved when potent vaccines prepared with the prototype strain were used. In fact the use of massive vaccination, together with the slaughtering of diseased and in-contact animals, as a control measure to contain the outbreaks has been successful. This is in agreement with the vaccine matching results obtained for the field strains, which eliminated the possibility that these outbreaks were caused by a variant not capable of being neutralized by potent vaccines properly applied in the susceptible population (PANAFTOSA PAHO/WHO, 2006).

Another important conclusion of the study is that none of these outbreaks were derived from poorly inactivated vaccines, in contrast to what was reported in other occasions (Beck and Strohmaier, 1987; König et al., 2001).

It is important to notice that although re-emergencies of the virus have been reported in zones already declared as FMD-free within a period of 7 years in the southern cone of South America, all variants clustered together in a unique lineage. This seems to point to a situation where active virus is present only in restricted niches and is expected to eventually reach populations with low immune levels, allowing the disease to be observed.

FMD in a previously disease-free region can cause a severe impact in the local and export trade of susceptible animals and their products. The emergence of such strains highlights the need to constantly monitor and characterize the isolates responsible for outbreaks, to be rapidly alerted so that appropriate control measures can be implemented. In this regard, the reappearance of FMDV in the southern cone of South America, and the molecular studies performed, reinforce the necessity to strengthen the serosurveillance activities, particularly directed to identify putative viral niches. The widespread application of phylogenetic techniques has permitted tracing genetic links among circulating viral strains. Such information, together with the results of serosurveys to establish potential viral niches, forms an important input in protecting against re-introductions of the agent in areas considered to be free of the infection. It should be noted that active surveillance activities can become, under certain circumstances, the only reliable tool to search for risk, as genetic-based tracing can only be applied when isolation of the agent has been achieved, and interpretations become conclusive when the variant characterization identifies an evident link with endemic or epidemic regions with reported viral circulation.

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