ANTIGENIC AND IMMUNOGENIC VARIABILITY OF THE FOOT-AND-MOUTH DISEASE VIRUS[1]

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SUMMARY

The high contagiousness of foot-and-mouth disease (FMD) and the great variability of the virus are factors that negatively affect the control of the disease. The disease-free countries therefore impose severe restrictions on the importation of animal products from countries affected by FMD. This situation thus aggravates the socio-economic problems of the affected countries and compels them to adopt strong measures to control and then eradicate the disease. In order for those measures to be effective, they must originate from the united determination of all those who, in one way or another, are dedicated to control the disease.

The control programs must unite the efforts of the livestock producers, the veterinary field services and the diagnosis, production and control laboratories, and must likewise maintain among them an active, continuous information regarding the characteristics of the viruses active in the field, the performance of the vaccines and the epidemiologic field situation. Additionally, FMD-control programs must put forth every effort to implement new techniques to study the antigenic and immunogenic characteristics of the virus and the changes in its nucleic acid for its better identification. Thus will the process of eradicating the disease be accelerated.

INTRODUCTION

Foot-and-mouth disease (FMD), one of the most important animal diseases because of its economic impact, is caused by a virus first isolated in 1897. Since then seven types with different antigenic and immunogenic properties have been identified and named: O Vallee, A Vallee, C Waldmann, SAT 1, SAT 2 and SAT 3, and ASIA 1. Their immunological differences are of such magnitude that animals recovering from one type are not protected against any other type.

Whithin each type virus groups or subtypes have been recognized which, although belonging to the same type, are not capable of conferring a solid immunity against other subtypes of the same type.

The viruses composing the subtypes are known as strains. They are usually designated by indicating the type and subtype to which they belong, and the place, country and year in which they were first isolated.

In order to remain free from the disease, the disease-free countries --North and Central America, Panama, the Caribbean countries, Chile, Australia, New Zealand, Japan, Great Britain and Ireland-- adopt severe restrictions on the importation of animal products from the infected countries.

The countries that are sporadically affected, such as Western Europe, also restrict imports.

This paper discusses the antigenic and immunogenic classification of FMD virus, intending to support the disease-control programs. The measures that should be adopted to reduce the economic impact of the diseases are also considered.

STRUCTURE OF THE VIRUS

The FMD virus is an enterovirus and is classified as a member of the *Picornaviridae* family.

In addition to the virion or complete virus with a sedimentation coefficient of 140S, three other particles have been found in suspensions of virulent virus: (a) the empty viral capsid, so named

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because it is devoid of ribonucleic acid (RNA) and is therefore not infectious, but has immunogenic properties similar to the 140S particle; (b) the 12S subunits of the capsid, and (c) the virus-infection-associated antigen (VIA) which is the viral polymerase with a sedimentation rate of 4.5. The virion has a single filament of RNA covered by a protein envelope of 12 capsomeres that form a symmetric icosahedric capsid having a diameter of 25nm.

The capsid is constituted of units of four structural polypeptides --VP1, VP2, VP3 and VP4. The VP1 is the most important of the four proteins. It is found on the outer part of the vertexes of the icosahedron, as has been demonstrated by treating the virus with trypsin and comparing the binding capacity of the IgM before and after the treatment. The antigenic and immunizing properties of the virus are closely linked to the integrity and sequence of the amino acids of the surface protein antigenic determinants.

VIRUS VARIABILITY

The FMD virus variation is due to its high rate of mutation (*9*), to genetic recombination, and to the selective pressure exerted in the field by virus replication in animals having inadequate levels of neutralizing antibodies. This gives rise to virus populations different from those that initiated the infection (*4*). These processes cause changes in the sequence of the nucleotides of the virus RNA, which would be transmitted to the capsid proteins, thereby modifying the sequence of the amino acids and, among other aspects, changing the antigenic and immunogenic properties of the viral antigenic determinants.

In the beginning, the variability studies relied on a determination of the viral antigenicity by complement fixation (*10*); that is, analysis focused on the capacity of the capsid surface antigenic determinants to bind with antibodies. The immunogenicity was later compared by measuring the antibody-inducing capacity by means of serum protection or serum neutralization tests in sera of the vaccinated animals (*3*).

The use of the ELISA (*1*) and of monoclonal antibodies (*8*) techniques, together with the implementation of modern biochemical techniques such as the T1 maps of the viral genomic RNA (*6*) and the electrofocusing of the capsid's proteins (*7*), led to more accurate studies of the virus and its full complexity.

The use of the new biological techniques such as the cloning and sequencing of the viral RNA (*5*) and the synthesis of small peptides with immunological relevance (*2*), in conjunction with the conventional diagnosis techniques and a more precise knowledge of the basic mechanism of virus variability, may enable researchers to predict the biological properties of the viruses that will appear in the field. The disease-control programs may thereby be able to anticipate the facts.

VIRUS CLASSIFICATION

The great variability of the FMD virus continually produces new strains that must be classified according to pre-established standards in order to avoid confusion. The current classification criteria originated from the need to support the disease-control programs. Therefore, the knowledge of the antigenic and immunogenic characteristics of the vaccine strains and field strains, and the importance of the latter, have to date been the fundamental elements for classifying the new strains.

The first classification standards were established in 1967 by the World Reference Laboratory at the International Symposium on Variants and Immunity, held in Lyon, France. Accordingly, the hyperimmune sera are titered by the 50% complement fixation method (CF_{50}) against the homologous and heterologous antigens. Based on the 50% complement fixation titers (CFT_{50}), the serological or antigenic relationships (r) and parentages (R) are established (Table 1), according to the following procedure:

$$r = \frac{CFT_{50} \text{ relative to heterologous antigen}}{CFT_{50} \text{ relative to homologous antigen}}$$

$$R = 100 \quad \sqrt{r_1 \times r_2}$$

r_1 = Relationship corresponding to the hyperimmune serum

r_2 = Relationship corresponding to the antigen

Limit of values:

	$R = r_1 \times r_2$	$R = 100\sqrt{r_1 \times r_2}$
1. Types	$\leqslant 0.01$	$\leqslant 10\%$
2. Very different subtypes	> 0.01 to 0,1	> 10 to 32%
3. Different subtypes	> 0.1 to 0.5	> 32 to 70%
4. Strains	> 0.5 to 1	$> 70\%$

TABLE 1. *Serological relationships (r_1 and r_2) of the most representative strains of FMD virus type A in the Southern Cone of South America*

Antigens (r_2)	Hyperimmune sera (r_1)				
	A_{24} Cruz.	A_{24} Arg.	A Venc.	A Arg/79	A Br/79
A_{24} Cruzeiro-Br/55	1.00	0.52	0.08	0.45	0.32
A_{24} Argentina/68	0.50	1.00	0.24	0.56	0.61
A Venceslau-Br/76	0.22	0.30	1.00	0.60	0.37
A Argentina/79	0.23	0.42	0.30	1.00	0.95
A Brasil/79	0.24	0.30	0.44	0.93	1.00

At the 1976 International Foot-and-Mouth Disease Symposium held in Lyon, it was proposed to disregard the parentages and to consider only the relationships. Moreover, it was added that a virus would thereafter be designated as a new subtype only when the strains were epidemiologically important, and only after it had been proved that immunological differences did exist between those strains and the strains used in producing the vaccine. These recommendations are not currently being applied, in so far as certain countries regard any new strain as a new subtype.

STUDY OF ANTIGENICITY AND IMMUNOGENICITY

Based on the recomendations of the World Reference Laboratory the CF_{50} method was utilized to determine the *r* and *R* relationships of the type A strains representative of the field samples found in the Southern Cone of South America, and isolated beginning in 1960 (Table 1). The immunological coverage was also established by serum neutralization of sera from bovines vaccinated and revaccinated with saponin-aluminum hydroxide vaccines; the sera were collected at 30 days post-vaccination (DPV) and at 30 and 90 days post-revaccination (DPR) (Table 2).

Analysis of the data in Table 1 indicates that all the strains studied, excepting A Arg/79 and A Br/79, should be classified as a different or very different subtypes. It is also observed that within a single strain the relationships correspnding to the serum (r_1) are, in many cases, quite different from those provided by the antigen (r_2).

In tests of this type, it is commonly observed that an antigen may have very high relationships with one strain and very low relationships with another, although both strains belong to the same subtype. It is also frequently observed that an antigen shows high and similar relationships with strains of different subtypes.

The number of strains included in a given subtype depends on the antigenicity of the reference strain for that subtype. The greater the antigenic spectrum, the larger the number of samples that will have serological relationships and will therefore be included in that subtype. The A Arg/79 strain has a broad spectrum and

TABLE 2. *Mean serum neutralization titers for FMD virus type A obtained with bovine sera at 30 DPV and 90 DPR*

	Sera from revaccinated cattle														
	A_{24} Cruz.			A Arg/68			A Venc.			A Arg/79			A Br/79		
	DPV	DPR		DPV	DPR		DPV	DPR		DPV	DPR		DPV	DPR	
Virus	30	30	90	30	30	90	30	30	90	30	30	90	30	30	90
A_{24} Cruzeiro	2.7	3.1	3.3	2.3	3.0	3.0	⩽ 1.4	2.3	2.3	⩽ 1.0	2.3	2.0	⩽ 1.4	2.1	1.9
A_{24} Argentina/68	2.4	3.6	2.9	3.0	3.3	3.2	⩽ 1.4	2.5	2.0	1.8	2.2	2.2	⩽ 1.5	2.4	2.2
A Venceslau	⩽ 1.6	2.4	2.8	2.2	2.4	2.7	3.0	3.5	3.3	2.4	2.6	2.6	2.3	2.9	2.6
A Argentina/79	⩽ 1.5	2.9	2.4	2.0	2.4	2.5	2.1	2.6	2.6	2.3	3.0	2.6	2.2	3.1	2.8
A Brasil/79	⩽ 1.5	2.9	2.4	2.0	2.4	2.4	2.3	2.6	2.5	2.4	2.9	2.7	2.2	2.8	2.7

DPV = Days post vaccination.
DPR = Days post revaccination.

therefore presents antigenic relationships with samples not having any epidemiological connection. All these aspects demonstrate the complexity of the variability of the virus and the difficulty of classifying it based only on antigenicity tests.

The immunological tests in Table 2 show that the five strains studied correspond to two different immunological groups. One group would comprise the first two strains and the second would include the last three. Vaccines formulated with a strain from each group would provide a broad immunological coverage because both strains are complementary.

The serum neutralization values obtained with the sera from the revaccinated cattle indicated that the revaccinations diminish the differences among the strains. These observations must also be taken into account for the classification of new subtypes.

ANTIGENICITY AND IMMUNOGENICITY OF THE FIELD STRAINS

The antigenic characterization of the field viruses conducted by the national laboratories in South America and by the Pan American Foot-and-Mouth Disease Center (PAFMDC), have shown that the persistence of the strains in the field varies from one strain to another. Laboratory and field studies have demonstrated that a given strain causes no more than one epidemic in the same area.

In the systematic characterization of the samples collected in a single region during the epidemic and inter-epidemic periods, it has been observed that the strains constantly modify their biological properties. Sometimes a virus gradually ceases to belong to a subtype. However, on other occasions striking antigenic differences appear suddenly.

A strain's speed and the size of the area over which it spreads are closely linked to the movement of animals and to the regions's physical features. In the Southern Cone of South America, the strains affect extensive areas. Conversely, in countries having important natural barriers and many independent regions, such as in Colombia, Ecuador and Peru, a given strain affects a small, well-defined area. Under these conditions different strains are frequently identified in the country's different regions over the same time period. On the other hand, in the Southern Cone a single strain is habitually predominant in an extensive area which often extends beyond a country's borders.

The constant mutation of the biological properties of the viruses in the field and the facility for maintaining the stability, antigenicity and immunogenicity in the laboratory enable one to distinguish whether a focus or an outbreak is the

consequency of a new field virus or escape of virus from the diagnosis, control, production or research laboratories. In such circumstances, the Viral RNA T_1 maps (*6*) are highly useful.

The strains identified within the same epidemic present small antigenic differences. During the inter-epidemic periods, greater differences are detected in viruses and new subtypes may be found. This phenomenon is due to the fact that the inter-epidemic periods are longer lasting and independent outbreaks originated by different strains will exist.

In samples collected in areas where the cattle are systematically vaccinated with vaccines of an acceptable quality, and therefore with antibodies specific to the vaccine strains, it has been observed that the virus tends to deviate antigenically and immunogenically from the strain utilized in the production. This is due to the continuous selection by the cattles' antibodies. The epidemic waves produced by strains having antigenic and immunogenic characteristics similar to those used in the vaccines clearly indicate that the vaccine is either of low immunogenic quality or is being inadequaly handled or there are susceptible populations.

VARIABILITY OF VIRUS TYPE A IN THE SOUTHERN CONE

An analysis of the behavior of the virus type A strains in the field, based on the antigenic and immunogenic studies conducted with samples collected in the Southern Cone, demonstrates that the variation of FMD virus was formerly closely linked to the development of the vaccine production methods.

The A_{13} Santos-Br/60 (*3*) was the first subtype identified. The virus' appearance was due to the production of antigen by the Waldmann method for vaccine preparation at the slaughterhouse in Santos, Brazil. Later, and also due to the production of Waldmann type vaccine, diagnosis revealed the subtypes A_{16} Belém-Br/59 and A_{17} Guarulhos-Br/59 subtypes. The three subtypes did not spread in the field, but because they were samples with great antigenic and immunogenic specificity they induced an immunity of limited coverage and yielded only slight protection.

In Argentina, the production of vaccines by the Waldmann method also led to the appearance of the A_{19} Suipacha-Arg/62 subtype. Like the other three subtypes, it possessed high antigenic and immunogenic specificity and likewise did not cause an epidemic. Another common characteristic of these subtypes is that they possess great replication capability and slight stability.

In a retrospective study conducted at the PAFMDC, involving an FMD focus in the province of Cordoba, Argentina, in 1961, an FMD virus similar to A_{10} Kemron was identified. It was also proven that a vaccine-production laboratory in Argentina was utilizing that strain to produce vaccine. The virus is used in Netherland for vaccine preparation. The same study identified the A_{25} Argentina/59 strain, which is also utilized by some vaccine-producing laboratories. The strain was sporadically isolated in the field during the 1959-1967 period. Nevertheless, the strains which predominated in the field in the Southern Cone from 1960 to 1975 belonged to the virus subtype A_{24}.

At the end of 1975 and during 1976, Brazil's southernmost state of Rio Grande do Sul suffered from an epidemic caused by the A Bagé-Br/76 strain belonging to the group comprising the A Venceslau-Br/76, A Arg/79 and A Br/79 viruses. That strain also caused an epidemic in late 1976 and early 1977 in Uruguay, and from August to November 1977, in Argentina. The Venceslau A type affected São Paulo and Paraná, Brazil, during that same period.

Another epidemic wave caused by the A Arg/79 strain was detected in Argentina from July 1980 to August 1981, as a consequence of the modification of the A Bagé-Br/76 type in the field. Strains similar to the Argentina/79 were also identified in Brazil and Uruguay.

CONCLUSIONS

The countries affected by FMD must eradicate the disease in order to prevent economic losses. Therefore they must adopt a series of measures originating in the common determination of the governments, the livestock producers and all those who in some way or another are involved in the agricultural and livestock-raising activity.

The FMD control and eradication programs must forge a close bond of interaction among the livestock producers, epidemiological surveillance and the diagnosis, production and vaccine-control laboratories.

With regard to the causative agent, the programs must be continually informed of the virus behavior in the field, and be familiar with the antigenic and immunogenic coverage of the vaccine strains for protection against the dominant field strains, in order to adopt timely measures when required.

In the countries of the Southern Cone where FMD is endemic, modern technology, which analyzes the virus' new aspects must collaborate with the existing structure to accelerate the control process and subsequent eradication of the disease.

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