ISOLATION OF FOOT-AND-MOUTH DISEASE VIRUS IN LABORATORY ANIMALS I. LIMITATIONS OF AVAILABLE INFORMATION

O.J. DEGREGORIO[1], V.M. VARELA-DIAZ[2]

[1]Facultad de Ciencias Veterinarias, Universidad de Buenos Aires
Avda. Chorroarín 280, Buenos Aires 1427, Argentina
[2]Pan-American Foot-and-Mouth Disease Center (PAHO/WHO)
P.O. Box 589, 20001-970 Rio de Janeiro, RJ, Brazil.

Summary. **Analysis of the available literature on the isolation of foot-and-mouth disease virus revealed that the inoculation of laboratory animals for diagnostic purposes has not been uniform. Studies carried out on the effectiveness of a variety of species differ from each other methodologically in several parameters which include the species, age, sex and strain of host as well as the dose, route of administration, and volume of the inoculum. The use of viruses adapted to culture or to different laboratory animal species represents another variable if it is considered that their infectivity may not be comparable to that of field strains. On this basis, the comparative susceptibility of the different species of laboratory animals to isolate Aphthovirus, and consequently, their diagnostic effectiveness in samples from bovines with natural infections, remains to be determined.**

Considering the great economic importance of foot-and-mouth disease to the livestock industry of South American countries, the availability of procedures for its rapid and accurate diagnosis, both in clinical cases and in carriers, is of prime interest. For this reason, a variety of laboratory animal species of diverse characteristics has been employed to isolate the Aphthovirus responsible for its etiology.

In general, work in laboratory animals has dealt with the pathogenicity (*3, 8, 10, 18, 19, 26, 28, 30, 32, 33, 35, 38, 42, 43*) and immunity (*14, 16*) of foot-and-mouth disease, as well as on the evaluation of diagnostic procedures (*1, 2, 4, 5, 7, 12, 15, 17, 20, 23, 24, 27, 31, 34, 36, 37, 39, 45*). Animals used for these studies included mice (*5, 7, 12-14, 21, 23, 36, 39-41, 43-45*), rats (*13,14,39*), guinea pigs (*1, 2, 5, 7,9,24,39-42*), rabbits (*9,15,20*), hamsters (*27,29,37*) and gerbils (*17*). Other species such as bovines (*7,22,23,36*), swine, dogs, cats and equines (*15*) as well as other systems, such as cell cultures (*4,7,23,31,36*) and chick embryos (*34*) have also been used.

The review of the literature on the isolation of Aphthovirus presented in this report suggested that the criteria for selecting different species of laboratory animals to inoculate them for diagnostic purposes were not based on standardized comparative studies. Accordingly, an analysis was made of the characteristics of the animals, viruses, inocula and experimental designs used, with the purpose of assessing the need to perform other studies on the subject.

Characteristics of animals

Firstly, it should be pointed out that the **number** of animals used, an essential fact to evaluate the implications of these studies, is reported in

Reprint requests to:
Pan American Foot-and-Mouth Disease Center (PAHO/WHO)

most publications (*1,2,5,7,9,12,17,22, 23,27, 36,40,43-45*), but not in many others (*4,13, 15,20,21,31,34,37,39,41,42*).

On the other hand, the **age** of animals varies widely throughout these papers, although it is recognized that differences in susceptibility have been associated with this characteristic (*43*). For example, reference was made to the use of newborn (*16*) or suckling mice of an unspecified age (*14*), or mice aged 1-2 days (*23*), 4-7 days (*43*), 4-8 days (*21*), 4-10 days (*13*), 5-7 days (*44*), 5-8 days (*5*), 6-8 days (*7,36*), or 7-10 days (*40*); mice aged one (*41*), three (*40*), or over five weeks (*39*) or 60-80 days (*12*); pregnant adults (*14*), females of 90 days (*5*), or 3 to 9 months old (*45*).

For other species, ages of rats were specified as 10-20 hours (*13*) or as suckling rats (*14,39*); for rabbits, merely newborns (*15*), or 45-day olds (*20*); and for hamsters, ages were given as 7 to 21 days (*37*) or 7 to 60 days (*35*). Gerbils used were 1-4 months old, suckling and adult animals (*17*); and calves were two years old (*22*). The ages of inoculated guinea pigs were 3 weeks (*7*), 3 to 10 weeks (*41*) or two to four months (*42*). Nevertheless, in most papers the option was made to refer to their **weight**, which was 450-500 g (*1*), 450-550 g (*24*), 464,15 ± 5,2 g (*2*) or 500-800 g (*42*).

The **sex** of experimental animals included female mice (*5, 12, 14, 39, 44*) and male calves (*7*) or guinea pigs (*18*). This characteristic was not mentioned in other publications (*1,2,4,13,15,20-24,29-36,40,42,43,45*).

The existence of variations in susceptibility to Aphthovirus infection among the different **strains** of laboratory animals has been demonstrated (*44*). Nevertheless, isolations have been made using more than 16 different *inbred and outbred* mice (*5,7,12,23,43-45*). On the other hand, this information was not reported for other species in a significant number of articles (*1,2,13-15,20-22, 31,36,39,40*), while in others (*7, 24*), Duncan-Hartley guinea pigs were used.

Characteristics of the inoculum

As with the above variations in the characteristics of the different laboratory animals, neither the inocula nor the technical conditions used for their administration have been uniformed.

Thus, different routes of inoculation have been used to isolate Aphthovirus. For instance, mice have been infected by intraperitoneal (*5,7,1214, 21,23,36,37,39,42-45*), subcutaneous (*13,21*), intracerebral (*21,40*) and intramuscular (*21,41,45*) routes, and rats were inoculated intraperitoneally (*13,14*). In guinea pigs, the routes included intradermoplantar (*1,2,39-42*), intralingual (*7,24*), subcutaneous (*15*) and intramuscular, intraperitoneal or digestive (*15, 42*). On the other hand, inoculations were intraperitoneal (*15*) or subcutaneous (*20*) for rabbits and by scarification (*27*) or by the intradermal, intraperitoneal and intracraneal (*35*) routes in hamsters. Gerbils were given intradermoplantar or intraperitoneal (*17*) inoculations, while in bovines, they were intradermolingual (*7,22,23,36*). In some papers (*34,37*), reference was not made to the route of inoculation.

The **dose** of virus which was inoculated is not indicated in most publications (*2,4,5,7,9,12-15, 17,20-24,27,31,36,37,40-45*). In others, the range constituted a wide spectrum: 10^4 to 10^6 ID_{50} in mice (*39*); or only a 10^{-4} dilution (*1*).

In most reports, no mention was made of the **volume** inoculated (*1,2,14,20,34,37,39,41,42*) and in the remainder, there was a great variability in this parameter. Thus, in mice given intraperitoneal infections, inocula consisted of 0.001-0.27 ml (*21*), 0.03 ml (*21, 23, 40, 43*), 0.04-0.03 ml (*13*), 0.05 ml (*7*), 0.1 ml (*5, 44, 45*), or 0.5 ml (*12*), while volumes of 0.01-0.27 ml (*21*) or 0.04-0.03 ml (*13*) were administered subcutaneously, and quantities of 0.001-0.05 ml 21 were applied intramuscularly. Inoculations in guinea pigs varied from 0.5-100 ml intraperitoneally (*15, 36*) to 0.1 ml by the intradermolingual (*36*) routes to as much as 0.3 ml by the intradermoplantar (*7*) route. In hamsters, inocula of 0.20 ml (*32*) or 0.25 ml (*29*) were administered intramuscularly, and in rabbits, from 0.25 to 1.0 ml (*9*) were given intraperitoneally. In

gerbils, 0.2 ml were inoculated by the intraperitoneal or intradermoplantar routes (*17*) while in bovines, the injections were intralingual and varied between 0.1 and 2 ml (*7,22,23*).

Characteristics of the virus

One of the principal limitations to interpreting the literature in terms of the comparative susceptibility of different laboratory animal species, and consequently, of the diagnostic effectiveness of this method, is associated with the **origin of the strain** of Aphthovirus used.

In certain studies, **modified strains** were used (*1,2,5,7,9,12,13,15,17,20,22,23,24,27,31,33, 34,36,37,39,40-45*). These had been obtained by passages in mice (*9,15,31,32, 39-41,44,45*), guinea pigs (*1,2,13,17,24,27,33,34,41,42*), hamsters (*37*), bovines (*1,2,7,12,13,22,33,36,39,41,44,45*), rabbits (*20*), piglets or chick embryos (*14*). In others, the strains were derived from cell cultures of bovine lingual epithelium (*7,23,36,45*), or of diverse origin (*31,43*), BHK21 (*12*), bovine kidney (*5,44*), or pig kidney cells (*44, 45*). In these studies, the **number of passages** of the virus ranged between 2 and 425.

If it is considered that the infectivity and virulence of Aphthovirus for different animal species is modified by repeated passage *in vitro* or *in vivo* (*6,11,25*), findings obtained with viruses which did not originate from natural infections are difficult to assess in terms of their applicability to field situations. Along these lines, it should be pointed out that only in some studies (*4,14,15,21,35,45*) were **field strains** employed. In most of them, the field viruses had been maintained through successive passages in mice (*15,21,45*) or alternatively, in rats and mice (*14*).

Only in two publications (*4, 35*) were results based on the use of field strains obtained directly from samples of infected bovines, although in one (*35*), findings were not differentiated from those obtained with adapted strains. This paucity of information does not seem consonant with the importance of foot-and-mouth disease virus isolations for epidemiologic and control purposes.

On the other hand, different viruses were used in published studies. These belonged to **type** O (*1,2,7,9,12-15,22,23,27,34,36,37,40,43, 4*), type A (*1,2,5,7,9,13-15,22,23,27,34,36,40,41,44*), type C (*1,2,7,9,13-15,17,22,24,27,31,36,39,40,42-44*), SAT1 and SAT2 (*7,23,36,40,44*), and SAT3 (*7,22, 23,36,44*), as well as type Asia 1 (*7,23,36,44*). In some publications (*4,20,21,45*), the type of virus employed was not stated.

Also, titrations of the various viral strains were carried out in mice (*7,9,12,21,27,43,45*), guinea pigs (*1,2,7,14,42*), tissue cultures (*7*), and bovine lingual epithelium (*7,9,22,23*). Nevertheless, these data were not provided in other studies (*4,5,13,17, 20,24,31,34,36,37,39-41, 44*). Furthermore, **virus effects** on the various animals were assessed by different techniques. These included the observation of clinical signs and lesions (*5,9,13,17,20,27, 37,40-42*); viral titrations of organs and/or tissues (*9,13,20,41*); quantification of antibodies (*4,12,14*); determination of ID_{50} in mice (*1,2,7,22,36,39,40, 43,44*) or of LD_{50} by the method of Reed and Muench (*12,15,21,24,36,37*).

CONCLUSIONS

This review points out important limitations on the usefulness of laboratory animals currently employed to isolate foot and mouth disease virus for diagnostic purposes. It is evident that studies must be devised and conducted to determine which species is most appropriate for future work in this field to enhance the efficiency and effectiveness of diagnosis. The studies must be designed to evaluate the responsiveness of different hosts to infection under identical physiological conditions and employ inocula that are standardized with respect to the viral dose, volumes and routes of administration. Finally, it appears to be particularly important that the Aphthovirus employed have not been adapted to any laboratory system.

ACKNOWLEDGEMENT

This study was submitted by the senior author in partial fulfillment of the requirements for the degree of Magister en Salud Animal de la Universidad de Buenos Aires.

REFERENCES

1. ARAMBURU, H.G. A comparison of different methods of inoculating guinea-pigs with the virus of foot-and-mouth disease. *J. Comp. Path., 59* (1): 43-47, 1949.
2. ARAMBURU, H.G. Inoculación de prueba en cobayos con virus aftoso: comparación de diferentes métodos. Inst.Nac.de Fiebre Aftosa. Minist. de Agric. de la Nación, 1949. (Pub. nº 9).
3. BROWN, C.C., OLANDER, H.J., MEYER, R.F. Pathogenesis of foot-and-mouth disease in guinea pigs using in situ hybridization. *Proc. Annual Mtg. US. Animal Hlth. Assoc., 93*: 321-323, 1989.
4. BUCKLEY, L.S., OSBORNE, R.W., PEREIRA, H.G. Laboratory diagnosis of foot- and-mouth disease and swine vesicular disease. *Bull. Off. int. Epiz., 88* (1-2): 128-129, 1975.
5. CAMPBELL, C.H. The susceptibility of mother mice and pregnant mice to the virus of foot-and-mouth disease. *J. Immunol., 84*: 469-474, 1960.
6. CARRILLO, C.E., RIEDER ROJAS, E., CAVALLARO, L., SCHIAPPACASSI, M., CAMPOS, R. Modification of foot-and-mouth disease virus after serial passages in the presence of antiviral polyclonal sera. *Virology, 171*: 599-601, 1989.
7. COTTRAL, G.E., PATTY, R.E., GAILIUNAS, P., SCOTT, F.W. Sensitivity of cell cultures, cattle, mice and guinea-pigs for detection of nineteen foot-and-mouth disease viruses. *Bull.Off.int.Epiz., 63* (9-10): 1607-1625, 1965.
8. CUNHA, R.G., EICHHORN, E.A. Influence of cortisone on susceptibility of adult mice to foot-and-mouth disease virus. *Am. J. Vet. Res.:* 149-151, 1954.
9. CUNHA, R.G., EICHHORN, E.A. Studies on rabbit-adapted foot-and-mouth disease virus. I.Propagation and pathogenicity. *Am. J. Vet. Res.:* 133-138, 1959.
10. DACORSO FILHO, P., CUNHA, R.G. Lesões observadas em coelho recém-nascidos inoculados com amostras de tres tipos de vírus de febre aftosa. *Bol. Soc. Bras. Med. Vet.,19*: 91-102, 1951.
11. DIEZ, J., MATEU, M.G., DOMINGO, E. Selection of antigenic variants of foot-and-mouth disease virus in the absence of antibodies, as revealed by an in situ assay. *J. Gen. Virol., 70*: 3281-3289, 1989.
12. FERNANDEZ, F.M., BORCA, M.V., SADIR, A.M., FONDEVILA, N., MAYO, J., SCHUDEL, A.A. Foot-and-mouth disease virus (FMDV) experimental infection: susceptibility and immune response of adult mice. *Vet. Microbiol., 12:* 15-24, 1986.
13. GARCIA MATA, E., PIZZI, L., ARAMBURU, H. El cultivo del virus aftoso en el ratón y en la rata blanca: su aplicación en los problemas de virología. *Gaceta Veterinaria, 13 (74)*: 1-8, 1951.
14. GARCIA MATA, E., PIZZI, L., ARAMBURU, H. Algunos aspectos de investigaciones con virus aftoso murinizado. *Gaceta Veterinaria, 14* (79): 223, 1952.
15. GARCIA MATA, E., FEDERER, K.E., PIZZI, L., ARAMBURU, H.G. Acción patógena del virus aftoso en neonatos de diferentes especies. *Gaceta Veterinaria, 17* (94): 57-64, 1955.
16. GARCIA MATA, E., FEDERER, K.E., PIZZI, L., MARCOVECCHIO, F.E., ARAGONA, J. Vacuna antiaftosa con virus adaptado a neonatos. En: *Congreso Argentino de Fiebre Aftosa*, Buenos Aires, Argentina, 14 - 15 de mayo de 1957. p. 233 237.
17. GIROUD, P., CIACCIO, G. Adaptation au mérion du virus aphteux. *C.R. Soc. Biol., Paris., 148*: 31-32, 1954.
18. GORHE, D.S. Inhibition of multiplication of foot-and-mouth disease virus in adult mice pretreated with Freund's complete adjuvant. *Nature, 216*: 1242-1244, 1967.
19. GRAVES, J.H., McKERCHER, P.D., CALLIS, J.J. Foot-and-mouth disease vaccine. Influence of the vaccine virus subtype on neutralizing antibody and resistance to disease. *Am. J. Vet. Res., 33* (4): 765-768, 1972.
20. GRIBANOV, V. Résultats de l'epreuve du vaccin antiaphteux VIEV préparé avec un virus adapté au lapin. *Bull. Off. int. Epiz., 43* (1): 632 635, 1955.
21. HEATLEY, W., SKINNER, H.H., SUBAK-SHARPE, H. Influence of route of inoculation and strain of mouse on infectivity titrations of the virus of foot-and-mouth disease. *Nature, 186* (4728): 99-911, 1960.
22. HENDERSON, W.M. A comparison of different routes of inoculation of cattle for detection of the virus of foot-and-mouth disease. *J. Hyg., 50* (2): 182-194, 1952.
23. HOUSE, C., HOUSE, J.A. Evaluation of techniques to demonstrate foot-and-mouth disease virus in bovine tongue epithelium: comparison of the sensitivity of cattle, mice, primary cell cultures, cryopreserved cell cultures and established cell lines. *Vet. Microbiol., 20*: 99-109, 1989.
24. HYDE, J.L., GRAVES, J.H. The comparative titration of foot-and-mouth disease virus inoculated into the tongue and foot pads of guinea pigs. *Am. J. Vet. Res., 24*: 99-100, 1963.

25. HYSLOP, N. St. G. Isolation of variants strains from foot-and-mouth disease virus in cell culture containing antiviral sera. *J. Hyg., Camb., 41:* 135-142, 1965.
26. KNUDSEN, R.C., GROOCOCK, C.M., ANDERSEN, A.A. Difference in protective immunity of the tongue and feet of guinea pigs vaccinated with foot-and-mouth disease virus type A12 following intradermolingual and footpad challenge. *Vet. Microbiol., 7*: 97-107, 1982.
27. KORN, G. Die erkrankung des goldhamsters, Mesocricetus auratus, au maul- und-klauenseuche. *Arch. Exp. Vet. Med., 6 (Suppl.)*: 36-37, 1952.
28. KORN, G. Die pathogenese und histogenese der maul-und-klauenseuche des goldhamsters, Mesocricetus auratus. *Arch. Exp. Vet. Med., 7*: 192-225, 1953.
29. LOMBARDO, H.J., MAYO, J., ABADIE, G., RIVENSON, S., SMOLKO, E.E. Adaptación del virus aftoso al hamster adulto. *Rev. Inv. Agrop. Ser. 4, 6* (8): 87-94, 1969.
30. LORD, R.D. Experimental infection of vampire bats with foot-and-mouth disease virus. *J. Wild. Dis., 22* (3): 413-414, 1986..
31. MACKOWIAK, C., LANG, R. Emploi des cultures de tissu dans le titrage du virus aphteux et la recherche des anticorps. *Bull. Off. int. Epiz., 49*: 99-105, 1958.
32. MAYO, J., LOMBARDO, J.H., SMOLKO, E.E., SEGURA, M., RIVENSON, S. Multiplicación del virus aftoso en roedores adultos previamente irradiados. *Rev. Inv. Agrop. Ser. 4, 3* (6): 57-69, 1966.
33. NAGEL, H.G. El comportamiento del virus aftoso en animales lactantes de diferentes especies. *Gaceta Veterinaria, 14* (76): 52-59, 1952.
34. NAGEL, H.C., PETERMANN, H.G. El comportamiento del virus aftoso en el embrión de pollo. *Gaceta Veterinaria, 14* (76): 73-78, 1952.
35. PALMA, E.E. Acción del virus aftoso frente al Cricetus cricetus (Hamster). En: *Congreso Argentino de Fiebre Aftosa*, Buenos Aires, Argentina, 14-16 de mayo de 1957. p.131-142.
36. PATTY, R.E., COTTRAL, G.E., GAILIUNAS, P. Comparative assay of foot-and-mouth disease virus in cattle, mice and cell cultures. *Bull. Off. int. Epiz., 63* (9-10): 1595-1606, 1965.
37. SCHMIDT FUNES, E. Receptivité du hamster au virus de la fievre aphteuse. *Bull. Off. int. Epiz., 43*: 756-760, 1955.
38. SCHUDEL, A.A., SADIR, A.M., ETCHEVERRIAGARAY, M.E., SAMUS, S., COLILA, O., RIVENSON, S. Susceptibility of South American non-primates to foot-and-mouth diseases virus. *Bull. Off. int. Epiz. 93* (11-12): 1345-1350, 1981.
39. SKINNER, H.H., HENDERSON, W.M., BROOKSBY, J.B. Use of unweaned white mice in foot-and-mouth disease research. *Nature, 169* (4306): 794-796, 1952.
40. SKINNER, H.H. Propagation of strains of foot-and-mouth disease virus in unweaned white mice. *Proc. Roy. Soc. Med., 44*: 1041-1044, 1958.
41. SKINNER, H.H., SMITH, I.M., HOLLOM, S.E., KNIGHT, E.H. Attenuated strains of the virus of foot-and-mouth disease. Studies in small animals with strains of common origin modified by different methods. *Arch. Ges. Virusforschung, 12*: 472-486, 1962.
42. SKINNER, H.H., KNIGHT, E.H. Environmental factors influencing the response of guinea-pigs to modified strains of foot-and-mouth disease virus. *Bull. Off. int. Epiz., 61* (9-10): 1-21, 1964.
43. SUBAK-SHARPE, H. The quantitative study of foot-and-mouth disease virus in unweaned mice. 1. Studies of various factors affecting quantitative analysis. *Arch. Ges. Virusforschung, 11*: 1-38, 1961.
44. SUBAK-SHARPE, H. The quantitative study of foot-and-mouth disease virus in unweaned mice. 2.Studies with additional mouse strains and comparison of some methods of titration. *Arch. Ges. Virusforschung, 11*: 39-63, 1961.
45. SUBAK-SHARPE, H. The effect of passage history, route of inoculation, virus strain and host strain on the susceptibility of adult mice to the virus of foot-and-mouth disease. *Arch. Ges. Virusforschung, 11* (3): 373-399, 1961.