

ISOLATION OF FOOT-AND-MOUTH DISEASE VIRUS IN LABORATORY ANIMALS II. COMPARATIVE SUSCEPTIBILITY OF SIX SPECIES

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Summary. The present findings demonstrate that the hamster is more susceptible to infection with foot-and-mouth disease virus than the suckling mouse, traditionally used for isolating this agent. Hamsters were more sensitive to the inoculation of Aphthovirus obtained from bovines with natural infections. The comparison was based on clinical manifestations, mean survival time, percent mortality, relationship between titer and mortality, and evolution of infection in weanlings. Following in decreasing order of susceptibility were suckling gerbils, rabbits and guinea pigs, while rats were refractory. The results are discussed in terms of their diagnostic implications for epidemiologic studies and disease control.

An analytical review of the bibliography on the use of laboratory animals for isolating foot-and-mouth disease virus revealed the limitations of current knowledge (7). On this basis, the need to carry out studies on the comparative susceptibility of different laboratory animal species under identical physiologic conditions, inoculated by the same route, with the same dose and volume of a field virus unadapted to any laboratory system was indicated.

Accordingly, this paper deals with the results of a study to assess the relative advantages and limitations of inoculating mice, rats, guinea pigs, rabbits, hamsters and gerbils to isolate Aphthovirus from epithelial samples obtained from cattle with natural infections.

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MATERIALS AND METHODS

Field samples

Epithelial samples were collected from the tongues of bovines infected naturally with foot-and-mouth disease virus, for use as inocula. Samples were placed in jars and covered with glycerine phosphate buffer. One of them (Nº. 1) was obtained in the locality of Pehuajó, Province of Buenos Aires, and others (Nos. 2 and 3) in the area of Chapaleufú, Province of La Pampa, Argentina. A 50 per cent dilution (1.4 ml) of each virus in Vallée medium was distributed in 2 ml, vials which had been sterilized under ultraviolet light for 15 minutes.

Antigen from epithelial samples

An aliquot (1.4 ml) of each epithelium was inoculated into roller bottles containing BHK₂₁ clone 13 culture cells in Eagle's maintenance me-

dium and incubated for 24 hours at 37°C. Following detection of cytopathic effect on the cell layer, bottles were frozen at -20°C for 24 hours. Subsequently, they were thawed in a steam bath at 37°C, centrifuged at 4°C for ten minutes at 2000 G's and the supernatant removed. All samples were kept refrigerated at 4°C until ready for use as antigen.

Viral titrations

Viral titers in microplates with preformed layers of IBRS-2 clone 17 cells were defined on the basis of the reciprocal of the viral dilution, expressed as logarithm base 10 (10^{-1} to 10^{-5}), that produced a cytopathic effect in 50 percent of cell layers (10^{50} (3)). The titer obtained was $10^{1.0}ID_{50}/ml$ for sample No. 1, $10^{1.5}ID_{50}/ml$ for sample No. 2; and $10^{0.85}ID_{50}/ml$ for sample No. 3.

Laboratory animal species

The species used were: CF1/Br mice (*Mus musculus*); Wistar Crw/Br rats (*Rattus norvegicus*); New Zealand white rabbits (*Oryctolagus cuniculus*); Cpz:Syr hamsters (*Mesocricetus auratus*); Hartley Hart guinea pigs (*Cavia porcellus*) and Cpz: Mon gerbils (*Meriones unguiculatus*).

Each group consisted of ten four-day old suckling animals, half of each sex. Three groups of each species were inoculated to characterize clinical signs and to register the appearance of mortality (expressed in hours post-inoculation) by sex and species. Suckling animals were kept with their mothers at all times.

For comparative purposes, similar studies were carried out using weaned animals to collect data using methods described for suckling animals. Species used were CF1/Br mice (*Mus musculus*); Cpz:Syr hamsters (*Mesocricetus auratus*); and Cpz:Mon gerbils (*Meriones unguiculatus*). Each group consisted of eight males, and their age was standardized at the third day after weaning. Three of these groups were inoculated for each species.

Animal inoculations

Animals were inoculated with the corresponding virus titer in a 0.03 ml volume, by the intraperitoneal route. In all cases, groups of animals were inoculated solely with diluent, and others were left untreated, for use as controls. Data on the occurrence of clinical signs and mortality were recorded over a five-day period.

Antigen from dead animals

Individuals which died after inoculation were eviscerated, and the skin, hair and heads were discarded. Subsequently, all animals were processed according to the technique described for mice (2). Part of each sample was used for typing and subtyping and the rest frozen at -20°C for subsequent inoculation of cell cultures to confirm the production of cytopathic effect by the virus.

Complement fixation

Typing of all samples was carried out by the complement fixation test using 50 per cent hemolysis (CF_{50}), as described elsewhere (2). Antigens prepared from suckling and weaned animals, and from bovine epithelium samples were used at the original 1:5 dilution. Antigens obtained from cell cultures were used at a 1:1 dilution.

Samples with anticomplement activity were diluted 1:10 in barbital buffer, clarified with 1 ml of chloroform and centrifuged at 2000 for 20 minutes, before repeating CF_{50} tests.

Statistical studies

The Student's t test (23) was applied to determine the possible existence of significant differences in mortality between species and by sex.

To estimate possible significant differences in morbidity and mortality between groups inoculated with various viral titers, studies of independence, X^2 tests (32) were used.

RESULTS

Typing of epithelial samples

Typing of bovine tongue epithelium samples by CF₅₀ confirmed that sample N^o. 1 corresponded to O₁ Campos-Brasil/58 virus, and samples Nos. 2 and 3 to C₃ Indaial-Brasil/71-Argentina/85 virus.

TABLE 1. Mortality by Aphthovirus in suckling laboratory animals.

	Virus O ₁ Campos		Virus C ₃ Indaial			
	Mortality (%)	Hours post-inoculation Median S.D.	Mortality (%)	Hours post-inoculation Median S.D.		
Hamsters	100	49.0	18.4	100	84.0	22.7
Mice	100	65.0	13.2	60	93.6	23.8
Gerbits	100	60.0	12.4	60	84.0	12.5
Rabbits	100	69.0	22.9	0	-	-
Guinea Pigs	20	40.0	12.4	0	-	-
Rats	0	-	-	0	-	-

Clinical studies and mortality in suckling animals

Mortality data obtained for species inoculated intraperitoneally with 0.03 ml of O₁ virus (mean titer 10³/ml) and with C₃ virus (mean titer of 10^{1.7}/ml) are shown in Table 1. No alterations were present in controls which received diluent alone or in normal suckling animals.

Clinical signs recorded in suckling animals after inoculating O₁ Campos virus were: for guinea pigs, minor signs, mainly weakness and hirsute hair, and for mice, posterior weakness and *ante-mortem* dyspnea. Only one hamster showed dyspnea and general weakness before death, while gerbils showed neurologic signs, mainly posterior paresia and dyspnea. One rabbit, which survived to 120 hours post-inoculation, presented paresia, posterior paralysis and generalized weakness before dying.

Clinical signs following suckling animal inoculations with C₃ virus (titer 10^{1.7}/ml) were slight in guinea pigs and mice, and consisted mainly of dyspnea, posterior weakness and hirsutism. Hamsters surviving more than 120 hours after inocula-

tion showed dyspnea and posterior weakness before death. Gerbils showed only signs of dyspnea.

In the third group of suckling animals inoculated intraperitoneally with 0.03 ml of C₃ virus (mean titer of 10^{0.85}/ml), no alterations or mortality were recorded in rabbits, gerbils, guinea pigs and rats, nor in diluent or normal controls. Mice and hamsters did not show clinical signs, although sudden death was recorded in all hamsters and one mouse.

Clinical studies and mortality in weaned animals

In weaned animals, mortality was 12.5 per cent for mice, and 100 per cent for hamsters following inoculation with O₁ virus (mean titer 10³/ml). In contrast, inoculated gerbils and control groups were unaffected.

Clinical signs in weaned hamsters included dyspnea, weakness, anterior and posterior paralysis, opisthotonus and purulent bilateral conjunctivitis, followed by loss of consciousness, and finally death. Suckling mice control groups showed no signs of infection.

Typing with viral antigens from infected animals

Typing results on laboratory animal samples are shown in Table 2. In general, the best CF₅₀ test antigen was that collected from suckling mice, followed by that from suckling gerbils, rabbits and hamsters. Similar results were obtained with samples from dead weaned animals.

In all cases, samples produced cytopathic effect in tissue cultures. Antigens of animal origin and those from tissue cultures retained their antigenic characteristics when frozen at -70°C for 90 days.

Statistical studies

Student's t tests on data from animals inoculated with O₁ Campos (titer of 10³/ml) revealed that only hamsters presented highly significant differences in mortality (P<0.0005) in relation to mice (t:3.536) and rabbits (t:3.094), while these were significant (P<0.0025) with respect to gerbils (t:2.134).

TABLE 2. Aphthovirus typing of samples from suckling laboratory animals.

	O ₁ (titer 10 ³ /ml)			C ₃ (titer 10 ^{1.7} /ml)			C ₃ (titer 10 ^{0.25} /ml)		
	Posi- tive	Direct Typing	Anti- comple- mentary	Posi- tive	Direct Typing	Anti- comple- mentary	Posi- tive	Direct Typing	Anti- comple- mentary
Mice	5	4	1	5	4	1	1	-	1
Hamsters	5	3	2	5	3	2	5	2	3
Gerbils	5	1	4	5	2	3	-	-	-
Rabbits	5	1	4	-	-	-	-	-	-

In relation to sex, male hamsters showed highly significant differences ($P < 0.0005$) in relation to male mice ($t: 3.069$) and rabbits ($t: 3.092$), and only significant differences ($P < 0.0025$) in relation to male gerbils ($t: 2.340$). Only female hamsters showed significant differences ($P < 0.0025$) with respect to mice ($t: 2.194$).

No significant differences among species or among their males were observed after inoculation with C₃ Indaial (titer of 10^{1.7}/ml). Only in the case of females, mice showed significant differences ($P < 0.0025$) in relation to hamsters ($t: 1.3979$) and gerbils ($t: 2.259$).

Morbidity and mortality at different viral titers

After inoculation of animals with the three titers of Aphthovirus, a value for $X^2 = 17.48$ was obtained when suckling mouse mortality results were analyzed, and a value of $X^2 = 20.32$ when morbidity was studied. On the other hand, these values were $X^2 = 0$ and 2.08, respectively, for suckling hamsters.

DISCUSSION

The present study provided evidence in support of the hypothesis that hamsters are more susceptible to inoculation by Aphthovirus obtained from bovines with natural infections than the com-

monly-employed suckling mice. In suckling hamsters, the *ante-mortem* time period was shorter and clinical manifestations more severe. Furthermore, it was observed that the onset of 100 per cent mortality was not associated to the viral titer of the inoculum and that it also occurred in weaned hamsters. In contrast, susceptibility was reduced in suckling gerbils, rabbits and guinea pigs while rats resisted infection.

For some species, clinical findings, mean survival times and mortality differed from those recorded in the literature (7). In the latter, the more pronounced manifestations were associated with inoculation of guinea pigs with viruses adapted to the homologous species (8,16); weaned gerbils, with virus adapted by 351 passages in guinea pigs (9); and weaned mice, with viruses modified by successive passages in suckling mice (4,13). These apparent discrepancies may be explained by considering that the Aphthovirus employed in this study were field samples, while other publications dealt with strains modified by repeated passages. Along these lines, it is recognized that the repeated passage of Aphthoviruses in tissue cultures or laboratory animals may modify their biological characteristics (7). Consequently, their titer, infectivity and virulence may increase in new substrates, and diminish for the original system.

In contrast, these differences in the severity of infections by field and adapted strains were not applicable to suckling or weaned hamsters, nor to

suckling mice or gerbils. This observation may be accounted for in terms of their having a high natural susceptibility to Aphthovirus, which does not allow the expression of differences between field and laboratory strains, as observed in this study and in others (9, 11-13, 16, 20). Similarly, these discrepancies were not seen with suckling rats or rabbits, an observation in agreement with their high degree of natural resistance to infection with field strains, as observed in this study and in other papers with modified viruses (5, 6, 16, 21).

The complement fixation test is widely used for the diagnosis and identification of foot-and-mouth disease viruses. However, in those cases in which the sample is inadequate, or the results inconclusive, it is deemed necessary to increase the available quantity of virus by means of passages in cell cultures, or in 2-7 day-old suckling mice (17). Accordingly, the greater susceptibility recorded for hamsters vis-à-vis other laboratory animals in the present study is suggestive of their usefulness in such diagnostic situations.

In general, the proposal to use hamsters to isolate Aphthovirus from field samples, particularly from highly contaminated epithelia, receives support from other considerations. These include the fact that the hamster is normally affected by fewer infectious diseases than other laboratory rodents (1) and the observation that these animals are used regularly for the selective isolation of leptospiras from contaminated samples (14, 15). Other characteristics of hamsters, such as their easy raising and management, render them suitable for production by laboratory animal facilities with an average infrastructure, as those which are distributed widely throughout Latin American and Caribbean countries (19).

Furthermore, recently weaned hamsters, whose susceptibility was comparable to that of suckling mice, clearly surpass the latter in factors related to management, breeding and development. These aspects are critical since they may distort expected morbidity and mortality indices, introduce significant deviations in trials, and consequently, condition results. Another advantage is that, because of their greater weight, they provide larger samples of infected tissues greater than suckling mice or hamsters.

In general, animal inoculations may be carried out in peripheral laboratories of low complexity, which predominate throughout Latin American and Caribbean countries (10, 18, 19). Viral replication in susceptible animals allow the quantities of available viral particles to increase, and accordingly, favours their viability during transit from field to central laboratories, for specialized studies. On this basis, it would be of interest to determine the relative value of hamsters and cell cultures for isolating Aphthovirus. The frequency of anti-complementary activity in samples from hamsters suggests the need to obtain data on the comparative effectiveness of other techniques used currently for typing Aphthovirus, in samples from different laboratory animal species.

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