AN OUTBREAK OF MURINE DIARRHOEA AND THE ISOLATION OF COXSACKIEVIRUS B_4[1]

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SINOPSE.- Liberto M.I.M., Nozawa C.M., Ono I.P.C., Vaz M.G.S., Carvalho T.C.G. & Linhares R.E.C. 1987. **[Surto de diarréia murina e isolamento de coxsackievírus B_4.]** An outbreak of murine diarrhoea and the isolation of coxsackievirus B_4. *Pesquisa Veterinária Brasileira 7(4):109-111.* Depto Virologia , Inst. Microbiol., Univ. Fed. Rio de Janeiro, Cx. Postal 68040, Rio de Janeiro, RJ 21944, Brazil.

Com o intuito de se identificar o eventual agente causador de diarréia aguda em uma colônia de criação de camundongos brancos suíços, materiais de animais doentes foram estudados através de técnicas virológicas. O homogenato de intestinos originários de quatro animais (21 dias) doentes foi submetido à clarificação e ultracentrifugação (100.000 g por 3 horas). O sedimento resultante foi processado por coloração negativa e observado ao microscópio eletrônico (EM) e inoculado em culturas de células HEp-2. Estas culturas desenvolveram efeito citopático semelhante ao dos enterovírus. O homogenato de células HEp-2 infectadas foi clarificado e inoculado em células LLC-MK_2 e em camundongos recém-natos por via intracerebral (branco suíço). Os animais desenvolveram paralisia espástica e as culturas de células LLC-MK_2 também apresentaram efeito semelhante ao dos enterovírus. Seções de cérebro congelado de animais inoculados, foram testadas por imunofluorescência indireta (FA) e mostraram fluorescência específica para coxsackievírus grupo B_4. O teste de neutralização (NT), em cultura de células, foi realizado com a suspensão originária de culturas de células LLC-MK_2 infectadas e confirmou o isolamento do vírus Coxsackie B_4.

TERMOS DE INDEXAÇÃO: Camundongo, diarréia, coxsackievírus.

ABSTRACT.- An outbreak of acute diarrhoea in a mouse breeding colony was studied. In order to identify a causal pathogen of the diarrhoeic process a virological study was carried out. A homogenate of intestine originating from four diseased young (21 days) animals was submitted to clarification and ultracentrifugation (100,000 g for 3 hours). The resulting sediment was processed for negative staining electron microscopy (EM) and inoculated into HEp-2 cell cultures. A cytopathic effect (CPE) resembling that of enteroviruses developed in these cultures. Homegenate of infected HEp-2 cells was clarified and inoculated in LLC-MK_2 cell cultures and newborn swiss mice. Newborn mice developed spastic paralysis and LLC-MK_2 cultures also presented enterovirus type CPE. Brain frozen sections of the inoculated animals were tested by Indirect Immunofluorescence (FA) and showed specific fluorescence for B_4 coxsackievirus. The neutralization test (NT) in cell cultures, carried out with viral suspension originating from LLC-MK_2 cultures, confirmed that coxsackie B_4 was the isolated virus.

INDEX TERMS: Mouse, diarrhoea, coxsackievirus.

INTRODUCTION

Murine enteroviruses do not cause diarrhoea in mice. They induce cardiac and neurologic diseases, as reported for the viruses of the enkephalomyocarditis (EMC) complex (Andrewes et al. 1978). Although these viruses are isolated from animal faeces they have not been implicated as causes of diarrhora. An important viral agent of mouse diarrhoea was described by Kraft (1957), as being one of the major causes of high morbidity and mortality in mouse breeding colonies, especially in young animals. This virus was named after its epidemiologic characteristics as Epidemic Diarrhoea of Infant Mice (EDIM), more recently known as mouse rotavirus (Flewett & Woode 1978). In spite of the age of the animals (21 days) it was thought that rotavirus could be the causal agent.

In this report, we studied an outbreak of mice diarrhoea. The possible aetiological role of the isolated viral agent is discussed in the light of the laboratorial findings.

MATERIALS AND METHODS

Clinical specimens of four or five pieces of resected small intestine containing faecal material originating from diseased young animals were homogeneized in 5 ml of PBS in a ten brock homogenizer. The homogenate was clarified by centrifugation and kept at -20°C until manipulation. Part of the clarified suspension was spun down at 10,000 g for 3 hours, after extraction with Freon (Union Carbide) (v/v).

Cell cultures. Cultures of continuous cell lines of HEp-2 and LLC-MK_2 were grown in MEM Eagle salt supplemented 5% bovine serum. For viral inoculation cell cultures were grown in 13 x 100 mm tubes at density of 5 x 10^6 cells/ml.

Newborn mice. Day-old mouse litters were inoculated intracerebrally with clarified homogenate of HEp-2 cell cultures which presented CPE.

Indirect Immunofluorescence Assay (FA). The conventional technique was used for FA in frozen sections of brain of newborn mice which were dying 5 days after inoculation.

Neutralization test (NT). The neutralization test was carried out in LLC-MK_2 cells in order to identify the isolate with specific anti-serum (Liberto et al. 1978).

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Electron microscopy (EM). Negative staining of clarified LCC-MK_2 homogenate, obtained after three cycles of freezing/thawing of the cultures presenting cytopathic effect, was performed on a "Formvar" coated 300 mesh copper grid with 2% fosfotungstic acid pH 6.5. This method was also used initially on the original clarified intestinal homogenate after ultracentrifugation at 100,000 g for 3 hours. A 301 electron microscope was used throughout the study.

RESULTS

The original intestinal material obtained from diseased animals did not show any viral particles by negative staining after concentration by ultracentrifugation. The material examined by EM was clean on three exhaustively examined grids. This same material negative by EM was appropriately diluted in tissue culture medium, clarified and passaged three times in HEp-2 cultures. At third passage, the monolayer presented cell rounding and picnosis observed 48 hours post-inoculation. The cytopathic effect (CPE) was similar to that shown by enteroviruses. Homogenate of HEp-2 cells with CPE was prepared by three consecutive cycles of freezing/thawing followed by clarification. A 10^{-1} dilution of the homogenate in tissue culture medium was inoculated in LLC-MK_2 cell culture and in three litters of newborn mice. LLC-MK_2 cell cultures also showed CPE similar to that observed in HEp-2 cells. Newborn mice inoculated intracerebrally presented spastic paralysis four days after inoculation.

The LLC-MK_2 supernatant presenting CPE was harvested and after negative staining showed several clumps of viral particles structurally similar to enteroviruses (Fig. 1).

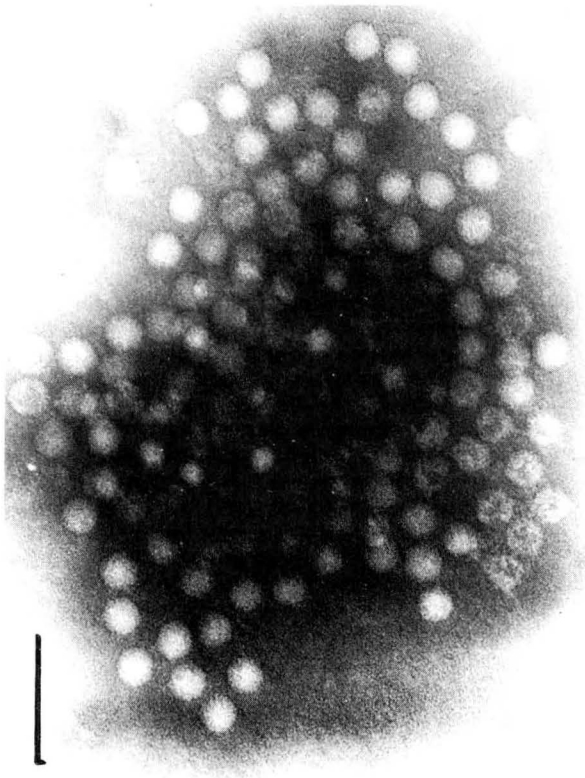

Fig. 1. *Viral particles observed in the homogenate of infected LLC-MK_2 cultures. Bar 100 nm.*

Combining the effect in cell cultures, spastic paralysis in mice and EM result, coxsackie B virus involvement was suspected. To confirm this hypothesis, isolated viral particles were identified by the neutralization test in LLC-MK_2 cells with specific anti-coxsackie B serum (B_1 to B_6). The neutralization test revealed a significant decrease in the infective titre when the isolate was treated with serum anti-B_4. Moreover, brain frozen sections of moribund newborn mice inoculated intracerebrally with homogenate of infected HEp-2 cells showed specific fluorescence for Coxsackie B_4 virus.

DISCUSSION AND CONCLUSION

It has been known for a long time that many viruses can be found in mouse intestinal tissue or faeces but in most cases without causing diarrhoea. These viruses include minute parvovirus, mouse adenovirus, murine cytomegalovirus, polyomavirus, reovirus 3 and Theiler's mouse enkephalomyelitis (picornavirus). However, two other viruses have been detected as real causes of outbreak of acute diarrhoea, namely mouse hepatitis virus (MHV), a coronavirus and epidemic diarrhoea of infant mice (EDIM) (see Carthew 1986 for review). Apart from Theiler's mouse enkephalomyelitis virus, no other murine enterovirus has been recorded as isolated from mouse faeces or intestinal material.

In this case, a close relationship is shown between an outbreak of diarrhoea in a mouse breeding colony and the isolation of an enterovirus. The analysis carried out in the original material resulted in the isolation of an enteroviruse-like viral particle detected by EM, although direct EM did not show any viral agent. This is possible because the number of viral particles in the original material was rather low (less than 10^6 particles/ml) even a after concentration by ultracentrifugation. However, after inoculation of the original material in cell cultures, an increase in viral particles was obtained. The CPE observed in HEp-2 and LLC-MK_2 cell cultures was compatible with enteroviruses. EM observation of viral particles in cell cultures homogenate strengthened the suspicion of the enterovirus presence in the original material. The size of the particles, 25-30 nm, and the structure in Fig. 1 undoubtly confirm the isolation of enteroviruses. The growth of the virus in mice suggested coxsackie enteroviruses, group B, as indicated by the spastic paralysis seen in dying animals. B_4 coxsackie was confirmed by specific fluorescence of newborn mice brain frozen sections and by the neutralization test in cell culture using specific coxsackie B_4 anti-serum.

Coxsackievirus is known to be a human pathogen causing mainly a vesicular disease called herpangina (group A). Group B coxsackieviruses are involved in diseases such as epidemic myalgia, aseptic meningitis and myocarditis especially in infants. Newborn mice experimentally inoculated with coxsackievirus develop paralysis and histopathological changes in several organs. Naturally acquired infection by coxsackievirus in mice has never been recorded (Crowell & Landau 1978, Liberto et al. 1987). The isolation and identification of coxsackievirus B_4 from mouse intestine suggests a possible role of this virus as a cause of acute diarrhoea in mice, yet again, never described before. Although initial material was exhaustively studied by EM no other viruses such as MHV or rotavirus were observed. However, complementary tests should be carried out in order to rule out the concomitant involvement of a rota and/or MHV with coxsackievirus. Bacteriological tests should also the carried out in order to eliminate the possibility of bacterial co-infection. There is no doubt that a long term study is necessary to clarify the involvement of coxsackievirus in mouse diarrhoea. However, this study shows that coxsackievirus can be found in diarrhoea in a mouse colony with high mortality rate. As far as we know, this is the first time that a human enterovirus has been isolated and identified in the mouse. It is not known how this virus entered the colony. However, being a relatively resistent virus, many ways of dissemination are possible. Al precautions must therefore be taken in order to avoid colony contamination that could have undesirable consequences.

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