

## EXPERIMENTAL INFECTION OF NORVEGIAN RATS (*RATTUS NORVEGICUS*) WITH RATPOX VIRUS

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*Summary.* — Young as well as adult free living Norwegian rats (*Rattus norvegicus*) were highly susceptible to intranasal, intradermal and natural infections with the ratpox virus belonging to genus *Orthopoxvirus*. Disseminated disease occurred in infected rats due to generalization of the virus to internal organs. In survivors, virus was excreted for a longer period of time (more than 1 month) in faeces and urine.

*Key words:* ratpox virus; Norwegian rat (*Rattus norvegicus*); pathogenicity; virus excretion

### Introduction

The transfer and maintenance of viruses in nature and their host spectrum are important ecological problems associated with the circulation of viruses among different animal species. Poxvirus isolations were reported from free living healthy animals, i. e. from rhombomys (*Rhomb. opimus*), susliks (Ladnyi *et al.*, 1975) and raccoons (Thomas *et al.*, 1975). In addition, poxvirus infection was demonstrated among laboratory rats (Krikun, 1975, 1977; Likhachev *et al.*, 1976; Marennikova *et al.*, 1978; Maiboroda and Lobanova, 1980). Likhachev *et al.* (1976) claimed that ratpox in laboratory rats represents a distinct entity caused by ratpox virus belonging to genus *Orthopoxvirus*, family *Poxviridae*. Ratpox virus, strain 012 Moscow 73 differed from ectromelia virus by several properties: by the morphology of pocks induced in the chorioallantoic membrane (CAM) of chick embryos, by a distinct pathogenicity for rabbits after intradermal and subcutaneous inoculations and by the susceptibility of a great variety of animals — rats, mice and rabbits (Maiboroda *et al.*, 1978, 1980). Free living rodents probably represent a link of poxvirus circulation in nature. The susceptibility of Norwegian rats to ratpox virus and their possible role in poxvirus circulation has not been cleared until now. The aim of the presented work is to investigate this problem.

### Materials and Methods

*Virus.* Ratpox virus strain IP-77 was isolated from lungs and kidneys of laboratory rats (breed August) in the course of an enzooecy. Based on the markers introduced for poxvirus differentiation, we classified the isolated strain as ratpox virus belonging to genus *Orthopoxvirus*,

family *Poxviridae*. The virus was passaged in the CAM of 12 days old chick embryos. Its infectivity titre in the 4th passage was  $7 \times 10^5$  PFU (pock forming units) per 0.1 ml.

*Animals.* Seventeen wild living Norwegian rats (*Rattus norvegicus*) of both sexes were used; from these 6 were adults caught in an animal farm; their 11 offsprings were held in captivity throughout. The young rats were infected either by intranasal route in mild ether narcosis (virus dose from  $7 \times 10^3$  to  $7 \times 10^5$  PFU/0.1 ml) or by scarification of the tail skin (virus dose  $7 \times 10^5$  PFU/0.1 ml). Adult rats were inoculated by natural route after having been distributed into cages left free following the death of young rats.

*Virus isolation.* Poxvirus was isolated from organ homogenates of infected animals, from their feces and urine by inoculation into CAM of chick embryos incubated at 37 °C. The isolated virus was identified according to the morphology of pocks on CAM, by microprecipitation in gel (utilizing 1% agar) with antisera prepared against vaccinia and ratpox viruses.

## Results

### Young animals

Intranasal inoculation of ratpox virus to 3 months old rats (in a dose from  $7 \times 10^3$  to  $7 \times 10^5$  PFU/0.1 ml) induced disease in 100% of animals within 4-5 days post infection (p. i.). After scarification of tail skin (inoculum of  $7 \times 10^5$  PFU/0.1 ml) the animals became ill within 7-8 days. Clinical manifestations were malaise, conjunctivitis, rhinitis, dyspnoe, skin eruptions and body distortion. Acute illness developed after intranasal inoculation; death occurred between 6 to 13 days p. i., rarely later on days 16-25 p. i. At autopsy, performed on days 6-13 p. i., haemorrhagic exsudate was found around the nozzle and in the nose cavity. Pox eruptions appeared on the skin of ears and tail. The most pronounced changes were observed in lungs and in gastrointestinal tract. Pneumonia was associated with multiple haemorrhages (Fig. 1); meteorism and distension of the stomach and intestine were accompanied with focal haemorrhages in the gut wall (Fig. 2). At later intervals necrotic foci were seen at the site of bleedings in the stomach and in the small and large intestines. The skin eruptions at these intervals were covered by crusts. In intranasally infected young rats the lethality was 100%.

In young rats infected by intradermal route the disease was similar, but less severe and revealed a prolonged course in the absence of acute symptoms. Occasionally mutilation of the paws was observed (Fig. 3).

### Adult animals

Clinical symptoms developed between 9-13 days since housing of adult rats in cages contaminated by young rats removed after death. Natural infection led to a disease with prolonged course and lethal outcome in the majority of cases occurring on days 10-35 as dated from the contact with the agent. Clinical symptoms and gross pathology were similar to changes found in laboratory rats infected by the same way and with the same virus dose.

Virus isolations confirmed the dissemination of poxvirus to internal organs. In young rats the highest concentration of the virus was found in lungs ( $10^5$ — $10^6$  PFU/0.1 ml of organ suspension) between 7-13 days after intranasal inoculation. Virus was detected in moderate concentrations in

liver, kidneys, spleen and testes ( $10^2$ – $10^3$  PFU/0.1 ml), while a very high concentration was found in rectum ( $10^6$  PFU/0.1 ml). In adult rats the virus was isolated from lungs, kidneys, liver and spleen from the 10th to the 35th day of the experiment. The highest titre was again determined in lungs ( $10^6$  PFU/0.1 ml). In both young and adult animals, virus was isolated from urine and faeces ( $10^3$ – $10^4$  PFU/0.1 ml). The excretion in urine and faeces was noted from 11 to 35 days p. i. No virus was isolated from the internal organs of an adult survivor sacrificed by day 70 p. i.

### Discussion

The studies in Norwegian rats confirmed their high susceptibility to ratpox virus isolated from laboratory rats. The pathology in infected free living rats was characterized by dissemination of the virus within the body, its subsequent reproduction and accumulation in internal organs to high titres and by a prolonged excretion of the virus in feces and urine. The high susceptibility of Norwegian rats to ratpox virus isolated from laboratory rats points to the importance of free living rodents in the spread of virus in question. The evidenced relatedness of ratpox and cowpox (Marennikova *et al.*, 1978) and the observations on circulation of poxviruses among free living rodents under natural conditions showed that the significance of laboratory as well as free living rodents in the ecology of poxviruses infecting domestic animals may be more pronounced as suggested till now.

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