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NEW OUTLOOK ON THE BIOLOGY OF COWPOX VIRUS

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Summary. — Analysis of cowpox outbreaks revealed an extremely wide range of virus pathogenicity including 9 orders of mammals. Recent serological and virological data support the hypothesis that wild rodents may be a natural reservoir of cowpox virus. Cowpox infection of humans occuring without any contact with infected cattle (registered in the U. K. and Poland) is especially interesting for medical and veterinary virology. Surveillance seems justified of the possible virus dissemination beyond its natural reservoir resulting in further infection of man and animals.

Key words: cowpox virus; natural reservoir; ecology of viruses

By eradication of smallpox all over the world the mankind have become liberated from the most severe poxvirus-caused disease. However, apart from the vaccinia virus which does not circulate in nature, there are another two orthopoxviruses pathogenic for human beings, namely monkeypox and cowpox. Therefore, it is obvious that more attention should be paid to their study. Some aspects of monkeypox infection are being investigated at present by a special WHO research project. New information on cowpox infection has been accumulated during the last decade. Some of these data are presented and discussed in this paper.

Before the seventies our knowledge on cowpox seemed to be definite and complete. The biological properties of *Orthopoxvirus bovis* had been extensively studied (Downie, 1939; Gispen, 1955; Rondle and Dumbell, 1962; Maltseva *et al.*, 1966; Baxby, 1975) and the generally accepted transmission pattern seemed very simple: humans (most frequently workers of diary farms) become infected by direct contact with cattle considered for natural reservoir of cowpox virus. In the past, outbreaks of cowpox were registered in several European countries: Germany, U.K., France, Austria and Poland (Gins, 1927; Dekking, 1964; von Kubin, 1970; Biernacka, 1972; Gibbs *et al.*, 1973; Biadala, 1976). Evidently, cowpox had been more frequent in previous years than nowadays. Statistical data of Gins (1927) on cowpox in Germany for the period of 1827—1837 (84 outbreaks for 10 years) support this assumption. To a certain extent, the reason could be in encountering clinically similar diseases of cattle caused by pseudocowpox (genus *Parapoxvirus*) and vaccinia viruses under diagnosis of cowpox infection (Gibbs *et al.*, 1970; Baxby, 1981). Our laboratory results obtained in 1968—1982 testify to the fact that outbreaks of diseases in the U.S.S.R. originally considered for

cowpox turned out to be vaccinia or pseudocowpox virus infections (Maltseva *et al.*, 1966; Voronenko *et al.*, 1971). Analogous observations were made by Dekking in Holland (1964) and others (Ahmedov *et al.*, 1960; Kornilova *et al.*, 1965). During last decades cowpox was most often registered in the Netherlands and England where there had been 36 cowpox oubreaks since 1947 to 1972 involving men (Gibbs *et al.*, 1973).

No explanation could be offered to the fact that several outbreaks of cowpox in cattle revealed no source of infection: they appeared spontaneously and frequently separated from each other by a period of several years. Dixon (1962) was the first who noticed this, calling such outbreaks "mysterious". Such observations, which had remained unexplained because they failed to fit into the accepted concept, began to accumulate in early seventies. In 1970 a pox-like disease affected 5 okapies (*Okapia johnstoni*) in the Zoo of Rotterdam (four adult animals and one calf). The initial symptoms — weakness, lethargy, poor appetite were followed by vesicular-pustular rash. The virus isolated from these animals resembled to cowpox virus (Zwart *et al.*, 1971). Another outbreak occurred among elephants in a travelling circus in Stuttgart in 1971 (Gehring *et al.*, 1972). The animals developed oedema of the skin of head, neck and hind limbs; then pustules appeared in the oral cavity and on skin folds of the tail. In one animal generalized infection developed: pustules grew confluent culminating in profound necrosis of the skin and mucous membranes. This animal died. German investigators who examined the outbreak claimed the isolate was vaccinia virus. However, subsequent studies at our laboratory of this as well as of okapi isolates along with results of Baxby and Ghaboosi (1977) who also investigated the causative agent of the pox-like disease in elephants, allowed to identify them as cowpox virus. In the meantime, a number of reports on pox-like diseases in elephants in circuses and Zoos has been published in GDR, Austria and Poland (Malecki and Zuchowska, 1980; Dathe, 1967; von Kubin *et al.*, 1975). The viruses isolated were identical with cowpox virus. Two outbreaks of pox-like disease in cheetahs were reported in different Zoos of U.K. occured in February 1977 and November 1978. The virus isolated did not differ from cowpox virus (Baxby *et al.*, 1979). In 1978 Thomsett *et al.*, and in 1983 Gaskell *et al.* reported isolation of cowpox virus from sick domestic cats.

In 1979 a number of papers had been published on pox disease of kangaroos, rhinoceroses, dolphins (McKenzie *et al.*; von Shaller and Pilaski; Geraci *et al.*). The laboratory diagnosis was based on the results of morphological study, brick-shaped virions and hyaline eosinophilic cytoplasmic inclusions were detected. Considering the type of the latter, cowpox virus was strongly suspected as the causative agent. The outbreaks described above were rather unusual and drew special interest but the source of infection was not traced and the origin of them was not determined.

For the first time we succeeded in tracing the source of infection when examining the outbreaks among *Carnivora* (*Felidae* family) and *Edentata* in Moscow Zoo in 1973 and 1974. The lions, black panthers, jaguars, pumas, cheetahs, ocelots, manuls and wild cats as well as giant anteaters contracted

the disease. Two forms of illness could be distinguished: fulminating pulmonary and dermal. About 40% of sick animals died (Surin *et al.*, 1974; Marennikova *et al.*, 1975; Marennikova *et al.*, 1976). The isolated virus was identified as cowpox virus (Marennikova *et al.*, 1977). Our further investigations showed that white rats supplied to the Zoo alive for feeding of some animals (pumas, ocelots, wild cats) were infected as well (Marennikova and Shelukhina, 1976). Subsequent laboratory study demonstrated the identity of the virus isolated from lungs and/or kidneys of apparently healthy white rats with the virus which caused the outbreak among the animals in Moscow Zoo. From the existing point of view, these results were quite unexpected since previously ectromelia was believed the only orthopoxvirus affecting rodents.

Retrospective epizootological analysis revealed that shortly before the outbreak in the Zoo an epizootic occurred among white rats in the Zoo supplier animal-farm. The majority of animals was affected. Pulmonary form of the disease prevailed and mortality rate reaching 30% (Marennikova *et al.*, 1978*a*). Probably by this reason the virus first isolated from the lungs of sick rats was termed "pneumotropic virus of the white rats" (Krikun, 1974). — Our investigations using phenotypic markers confirmed the identity of the above mentioned virus with the virus isolated from apparently healthy white rats as virus carriers as well as with the isolates from carnivora of the Zoo — the elephantpox and okapipox viruses which had been described above including the isolates from big gerbil (*Rhombomyc opimus*) of Turkmenia and of a polish farmer (to be described below).

Comparative study of the six isolates listed above and of six strains of cowpox virus (including reference) isolated and studied earlier in England and in the Netherlands (Downie, 1939; Gispen, 1955; Baxby, 1975) showed that all 12 viruses under study were subspecies (strains) of *Orthopoxvirus bovis*. Nine strains differed from the Brighton reference-strain in the upper temperature limiting virus multiplication on CAM and two of them in the pathogenicity for laboratory rodents. Data on significant intraspecies variability of cowpox virus were reported by Baxby (1975) who compared 18 strains of the virus. The identity of the Moscow isolates from white rats and carnivora to the reference cowpox strain Brighton was confirmed by prophiles of polypeptides induced by these viruses in infected cells (Harper *et al.*, 1979).

The studies outlined above have introduced new facts on the ecology of cowpox virus. First, it is the wide spectrum of pathogenicity for the animals belonging to various taxonomic groups. Fig. 1 demonstrates the host range of cowpox virus. According to the data available, cowpox virus can affect animals of 9 (out of 18) mammalian orders.

Second, the persistence came to light of cowpox virus in white laboratory rats (family *Muridae*, order *Rodentia*). This finding was reproduced experimentally (Shelukhina *et al.*, 1979; Maiboroda and Lobanova, 1980). Its epidemiological significance had been proved not only in the Zoo outbreak but in two additional outbreaks, which occurred within 7 months in the farm as well as in the Zoo. On the-basis of these data we suggest that persistence of

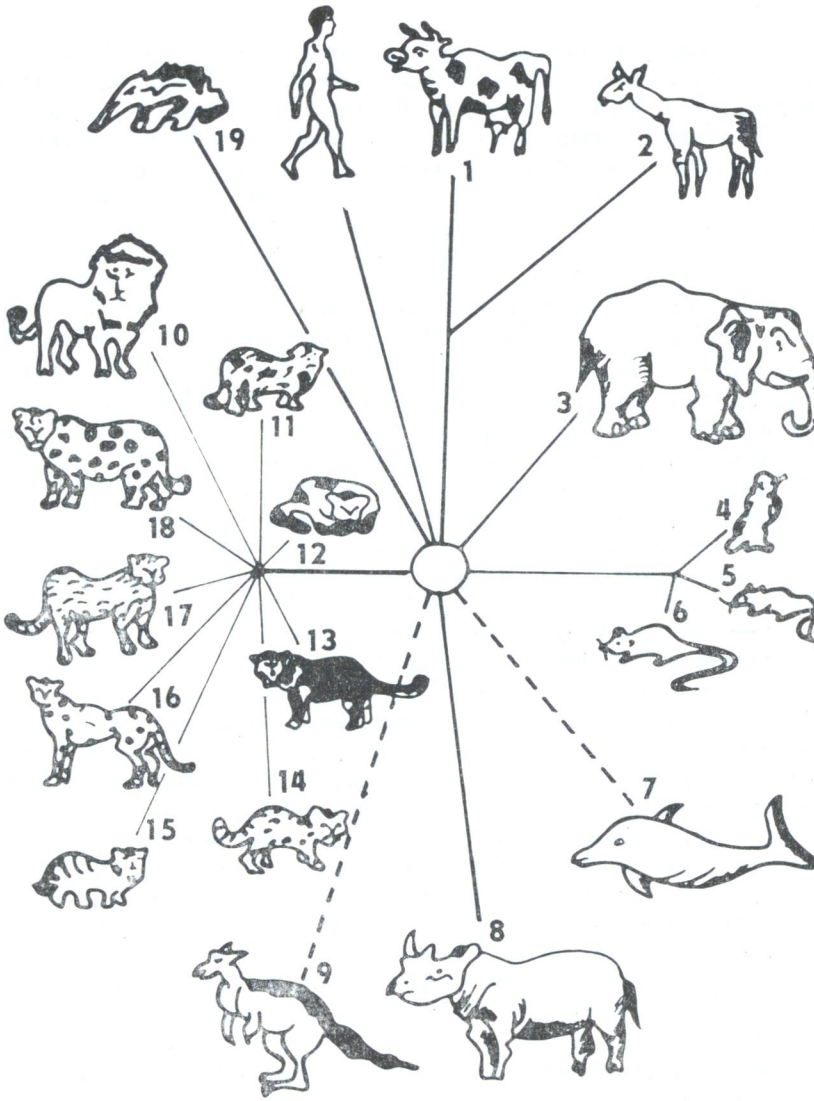

Fig. 1.

Host range of cowpox virus

Orders of *Mammalia* — clockwise from Primates-Homo sapiens (man): *Artiodactyla — Vacca* (cow) (1), *Okapia johnstoni* (2); *Proboscidea — Elephas maximus* (elephant) (3); *Rodentia — Citellus fullvus* (yellow souslik) (4), *Rhombomys opimus* (big gerbil) (5), *Rattus albus* (white rat) (6); *Cetacea— Delphinus delphis* (dolphin) (7); *Perissodactyla — Diceros simus* (white rhinoceros) (8); *Marsupialia — Macropus canguru* (gray kangaroo) (9).

Species of family *Felidae*, order *Carnivora* — clockwise from man: *Panthera leo* (lion) (10), *Felis bengalensis* (leopard cat) (11), *Felis domesticus* (domestic cat) (12), *Panthera pardus* (black panther) (13), *Felis pardalis* (ocelot) (14), *Felis manul* (Pallas's cat) (15), *Acinonyx jubatus* (cheetah) (16), *Felis concolor* (puma) (17), *Felis onca* (jaguar) (18).

the virus in rodents is one of the mechanisms of maintenance of cowpox virus in the nature. High susceptibility of rodents to cowpox virus, their wide distribution all over the world and their various biological links with animals of the other species testify to the favour of this hypothesis.

White rats are laboratory animals not living free in nature; therefore the above statement should be proved in wild rodents. With this in mind we (in collaboration with Dr. Levy) studied the susceptibility of wild grey rats (*Rattus norvegicus*) to the virus isolated from laboratory rats. Wild rats were found highly susceptible to this virus when inoculated intranasally. Inoculation of 10^6 PFU of the virus caused pulmonary form of the disease with mortality reaching 66%. All healthy rats housed together with sick animals got infected. Susceptibility of young and adult rats to the same virus was demonstrated independently by Maiboroda (1982).

However, the most reliable proof of the role of rodents would be a natural reservoir of cowpox virus as obtained by examination of wild rodents under natural conditions. Extensive serological examinations had been carried out in wild rodents in remoted desert and semidesert areas of Turkmenia which is rich of rodents. Antihaemagglutinins and virusneutralizing antibodies to orthopoxviruses have been detected in 4 out of 9 rodent species (*Rhombomys opimus*, *Citellus fulvus*, *Meriones meridianus* and *Meriones libicus*). Antibodies prevailed most frequently in big gerbils and yellow sousliks: in 18.6% and 15.3%, respectively (Ladnyi *et al.*, 1975). Three virus strains indistinguishable from cowpox were isolated from the organs (kidneys and spleen) of big gerbil and yellow souslik as a result of investigation of 1275 rodents (Marennikova *et al.*, 1978*b*). It should be emphasized that to exclude laboratory contamination virus isolation was carried out in a laboratory where a work with poxviruses has never been done before.

It was shown that both species of rodents were gighly susceptible to experimental infection with cowpox virus. The disease was clinically manifested and followed by high mortality (from 30% to 100%). The virus persisted in visceral organs of rodents convalescents: it was continuously isolated from kidney and testicular tissue till the 5th week (observation period). These data substantially support the assumption that wild rodents of various species may serve as natural reservoir for the cowpox virus, and cattle as well as men should obviously be considered as occasional host. In connection with this, the question may arise whether the term "cowpox" is justified enough. The fact of permanent circulation of the cowpox virus in rodents under natural conditions explains the mechanism of infection in animals of circuses and Zoos as well as the prolonged gaps between outbreaks in cattle and sudden relapse of the disease. Finally, this hypothesis offers an explanation to another fact, which previously could not be satisfactorily interpreted.

Edentata — *Myrmecophaga tridactyla* (giant anteater) (19). Names of animal species (in Latin and English) affected by cowpox are given after the names of orders. Diagnosis of cowpox was confirmed virologically. Interrupted lines: orders of animals with laboratory diagnosis of cowpox based on morphological data only.

Human cowpox was usually regarded for an occupational disease of diary farmworkers (Baxby, 1975; Baxby and Osborne, 1979). However, in 1975 three separate cases were described of human cowpox where contact with cattle was not established (Brit. Med. J., editorial). In 1977 Baxby described 7 cases of human cowpox in England. No cases of bovine cowpox were detected in the vicinity and serological survey gave results which did not suggest cowpox in cattle. Two cases of human cowpox were examined by polish scientists (Woinarowska, Bochenek, Dziok) in collaboration with us in 1977. Both cases occured in the same locality of Poland during grain harvesting in which women took part. Diagnosis was confirmed by virus isolation and its identification — in the first case, and by retrospective serological data — in the second one. Virus isolated from eye discharge was characterized as *Orthopoxvirus bovis* by a number of phenotypical markers as well as by analysis of viral DNA cleaved by endonucleases Hind III, Xho I and Sma I. Disease of cattle caused by poxviruses was not registered at that particular time neither in this locality nor anywhere else in Poland. This fact was also confirmed by serological examination of cows husbanded by the two patients. There were no recent vaccinees in the vicinity of sick women. The last known case of cowpox was registered in Poland in 1967 (Biernacka, 1972).

Contact with rodents can not be excluded either in cases just described or in some other cases mentioned above. Yet such a contact should not necessarily be direct as observations made by Maiboroda (1982) showed that alive virus could be excreted by sick wild rats in urine and faeces. The data in this paper suggest a view quite different from the traditional on ecology of cowpox virus and are of practical importance for medical and veterinary virology. A correct concept of cowpox transmission would create necessary surveillance due to the possibility of virus spread beyond the natural reservoir and infection of humans and animals. Though at present there is no valid reason to believe that the pathogenicity of this virus and its contagiousity have changed to any extent since Edward Jenner's time, it would be wrong to ignore the potential risk of cowpox infection especially under conditions of discontinuation of the smallpox vaccination over the world.

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