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MONKEY POX IN HUMANS: CURRENT RESULTS

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Summary. — The presented studies were performed at the WHO Collaboration Center for Smallpox in Moscow in the framework of the WHO monkey pox project. The authors recommend improved methods for rapid detection of orthopoxvirus antigen, namely passive haemagglutination (PHA) using dried stable red blood cells and ELISA in order to provide more rapid and efficient laboratory diagnosis under field conditions. Independent serologic diagnosis of monkey pox by ELISA-adsorption (ELISA-A) was proved of value for epidemiological studies and for detection of inapparent infections. The application range of the latter technique and its limitations were also determined.

Key words: *monkey pox*; *PHA*; *ELISA-adsorption*; *orthopox viruses*; *antibodies*

Introduction

As discovered in 1970 monkey pox clinically similar to smallpox may occur in humans (Marennikova *et al.*, 1971; Marennikova *et al.*, 1972; Ladnyi *et al.*, 1972). This appeared important not only for performing the smallpox eradication program, but also for elaboration of the posteradication strategy. Thus, timely deciphering the nature of monkey pox assured the adequacy of the chosen measures and finally promoted the successful completion of the smallpox eradication program.

The relevance of this disease in the posteradication period was determined by two major considerations. First there was some concern whether the low transmission of monkey pox from man to man — that presently discriminates it from smallpox — will remain limited and would not increase owing to the decrease of communal immunity of the population after smallpox vaccinations ceased. Second, some variations in properties of the virus itself which may lead to enhanced contagiousness of the disease cannot be ruled out. The insufficient knowledge of monkey pox in humans and of its potential danger made WHO to continue the surveillance in Central Africa (in Zaire) from 1982 to 1986 as a part of a special project on complex monkey pox investigations. The present paper describes the results of the virological and serological studies carried out by the WHO Collaboration Center for Smallpox and other poxviruses in Moscow from 1984 to 1986* to provide a laboratory service for the project.

Materials and Methods

Diagnostic samples. Samples from patients suspected to have monkey pox were obtained from Zaire Republic and Central African Republic (CAR) were skin lesion contents and/or blood serum. The latter was also collected from contact subjects. The material was collected by the WHO Surveillance teams or by local medical personnel. The samples were shipped by plane, but it took from 2 weeks to 11 1/2 months until they arrived to our laboratory.

Virus isolation. The virus was isolated from the skin lesion content by inoculation of chorionallantoic membrane (CAM) of 12-day-old chick embryos (WHO, 1969). Since penicilline and streptomycine only appeared insufficient to prevent bacterial contaminations, gentamycine at a concentration of 0.2 μg/ml was added. For electron microscopy, the skin samples were applied on a grid and treated by negative contrasting (Cruickshank *et al.*, 1966); the preparations were examined in the microscope JEM-100 CX II.

Serologic tests. Antibodies against orthopox viruses were detected by ELISA (Marennikova *et al.*, 1984). Identification of species specific antibodies was carried out by ELISA-adsorption (ELISA-A) according to the level of residual antibodies to vaccinia and monkey pox viruses after adsorption of each serum to CAM-grown vaccinia virus (Maltseva *et al.*, 1984; Hutchinson *et al.*, 1977). Antibodies to varicella-zoster virus were detected by ELISA using an antigen which had been prepared in human diploid embryo lung cells and absorbed to plates (Flow Laboratories).

Further arrangements are described below for the sake of convenience.

Results

The results of diagnostic tests are summarized in Table 1. Electron microscopic examinations of the 301 samples from patients (skin lesion content) suspected to have monkey pox revealed poxvirus virions in 96 samples (31.9 %). Monkey pox virus was isolated from 73 samples (23.1 %). The lower percentage of virus isolation as compared with electron microscopic findings can be explained by the possible presence of other poxviruses (Tana pox, contagious mollusc) which cannot grow on chick embryo CAM but are morphologically indistinguishable. It cannot be ruled out that in single cases (careless collection of material, small quantity of the material) the failure to isolate the virus could be due to the loss of the viability of the virus during shipment.

Virus isolation results showed that in human monkey pox, alike to smallpox, the virus can be isolated from skin lesions of all types (papule scraping, fluids from vesicles and pustulae, crusts) starting from day 2 to day 21 of the disease (observation time). Serologic tests confirmed the diagnosis of monkey pox in 25 % of cases. Serology was used either in addition to above-mentioned techniques or as the basic diagnostic method (if for some reason no material from patient's skin lesions was available or the study was carried out retrospectively). The value of retrospective serologic examination can be illustrated by a case when monkey pox was diagnosed in a girl by ELISA-A carried out 5 1/2 months after she had fever with rash. These data, in turn, allowed us to establish that in endemic area (Zaire Republic) monkey pox was transmitted among humans over 3 generations and not only over 2 as previously assumed.

The antibodies in monkey pox patients as detected by ELISA appeared early and reached high titres even within the first five days. Their maximal

* From January 1984 till December 1986

Table 1. General results of diagnostic studies

Material tested	Virions detected by electron microscopy		Monkey pox virus isolates
	pox group	herpes group	
The content of skin lesions of patients suspected to have	96/301 (31.9 %)	35/301 (11.6 %)	73/316 (23.1 %)

Material tested	Antibodies to viruses detected in the tests			
	ELISA-adsorption		ELISA	
	monkey pox virus	vaccinia virus	unidentifiable antibodies (not distinguishing vaccinia virus from monkey pox virus)	varicella-zoster virus
Sera of patients suspected to have monkey pox or having had contacts with a ti'er*	78**/311 (25 %)	17/311 (5.5 %)	18/311 (5.8 %)	46/196 (23.5)

Notes: numerator — number of positive results, denominator — total number of samples tested.
* The group consisted of 183 subjects who had a direct contact with monkey pox patients.
** Including 11 sera for which the case was diagnosed according to the difference in the titres of antibodies to monkey pox and vaccinia viruses (see the text).

accumulation was observed on day 14 followed by a slow decrease of the antibody level (Fig. 1). After 3 1/2 months (observation time) the titre was still high. In 11.3 % (Table 1) of the subjects examined their sera contained either antibodies against vaccinia virus or the so-called unidentified antibodies (orthopoxvirus antibodies not distinguishing monkey pox virus from vaccinia virus in ELISA-A). Such results could not provide the basis for diagnosis, neither could they disprove it. Thus, for example, in 2 patients earlier vaccinated against smallpox (one of them appeared to have antibodies to vaccinia virus and the other had unidentifiable antibodies) monkey pox virus was isolated from skin lesions which is a doubtless evidence that these subjects had been infected with given virus.

As far as unidentifiable antibodies are concerned, some tests with paired sera taken from the same patient suggested that the time after the onset of the disease is one of the factors responsible for the species-specificity of antibodies. In the sera taken from monkey pox patients within the first 2 days of the disease and also at the late convalescent period (after week 19 in our

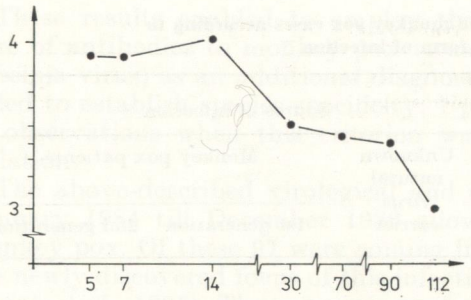

Fig. 1.
Detection of serum antibodies in monkey pox virus-infected patients
Abscissa — days of disease; ordinate — antibody titres in ELISA (log. of dilution reciprocals).

bservations) unidentifiable antibodies occurred more often. On the other a unidentifiable antibodies were detected in monkey pox patients that been previously vaccinated against smallpox.

These observations called for an experimental study of probable causes of the above-mentioned results in ELISA-A tests. The experiments were carried out in cotton rats (*Sigmodon hispidus*) aged 3—4 weeks. The animals were intranasally infected with vaccinia virus or one of the cowpox virus biovariants — carnivor pox virus — in order to produce orthopox virus infection. The development of specific antibodies has then been studied with the help of ELISA-A. After 4 to 6 weeks the animals of each group were reinfected with a heterologous virus; those that had undergone vaccinia virus infection were inoculated intranasally with carnivor pox virus and vice versa. The results of these experiments generally confirmed our suggestion. On

Table 2. Serological diagnosis based on the difference in the titres of antibodies to the virus that caused the infection and a closely related orthopox virus

Tested materials	Number of subjects examined	Of these with relation of ELISA titre of the virus that caused the infection to the titre to heterologous (closely related) virus		
		at least 3 times as high	equal	lower
Experimentally infected animals* in whose serum species-specific antibodies to the virus that caused the infection were detected	47	34 (72.4 %)	13 (27.6 %)	0
Monkey pox patients** who were found to have species-specific antibodies to monkey pox virus	78	54 (69.2 2 %)	24 (30.8 %)	0

* Cotton rats intranasally invected with vaccinia virus and cowpox virus.
** Inapparent infections included.

Table 3. Distribution of revealed monkey pox cases according to the source and form of infection

Form of infection	Number of cases revealed	Source of infection		
		Unknown natural virus carrier	Monkey pox patients	
			1st generation	2nd generation
Clinical infection	87	64 (73.5 %)	21 (24.1 %)	2 (2.4 %)
Inapparent infection	16	4 (25 %)	12 (75 %)	0
Total	103	68 (66.0 %)	33 (32.1 %	2 (1.9 %)

infection with an orthopox virus (vaccinia virus), group-specific (genus orthopox virus) antibodies increased in all the animals and species-specificity was unidentifiable at early post-infection period in 37.5 % of animals. This index was significantly decreased starting week 3. The regularity was even more marked with carnivor pox virus.

The results of successive infections with 2 orthopox viruses appeared to depend both on the dose and on the species of the virus used for primary infection and superinfection. Species-specificity of antibodies after superinfection was either unchanged or became homologous to the virus used for superinfection. In both cases, however, successive contacts with 2 closely related orthopox viruses resulted in an increase in the percentage of unidentifiable antibodies.

It should be noted that whatever was the cause of the appearance of unidentified antibodies they make a precise diagnosis impossible in a significant percentage of cases. Therefore, both additional serodiagnosis criteria are needed and ways to improve the presently employed technique should be sought. The ratio of the titre of antibodies to monkey pox virus causing the disease and of the titre of antibodies to a closely related orthopoxvirus was determined in both the experimentally infected animals and in convalescent monkey pox patients. The results were analysed only with those sera in which species-specificity of antibodies was definitely detected by ELISA-A. The titre of antibodies in monkey pox patients in 69.2 % of cases was 3 and more times as high with homologous virus as with vaccinia virus. In 30.8 % of cases the titres with homologous and heterologous viruses were equal. In experiments with cotton rats infected with vaccinia virus or cowpox virus the titre of antibodies with homologous virus appeared to be higher in 72.4 % of cases; the titres with homologous and heterologous virus were equal in 27.6 % of cases; inverse antibody relationship was never observed (Table 2).

These results enabled to employ the difference in antibody titres (the titre of antibodies to monkey pox virus at least 3 times as high as that to vaccinia virus) as an additional diagnostic criterion in cases when ELISA-A failed to establish species-specificity. Up to present, we have had a number of observations when this criterion was confirmed by monkey pox virus isolation.

The above-described virological and serological studies carried out from January 1984 till December 1986 allowed us to reveal 103 patients with monkey pox. Of these 97 were coming from Zaire Republic and 6 were from the newly discovered focus of this infection among Pygmies in CAR (Khodakevich *et al.*, 1985). The comparison of our results with epidemiological data obtained by WHO surveillance teams has shown the following. Overwhelming majority of monkey pox patients were the so-called primary cases, i.e. the subjects infected via contact with an unknown natural carrier (1st generation). Secondary cases accounted for 24 % (they are highly likely to have been infected by a 1st generation monkey pox patient). In two cases a 3rd generation of man-to-man transmission has been found. From 183 healthy subjects who had had a contact with a monkey pox patients (from Zaire Republic and CAR) the serologic tests revealed inapparent infection in 16 subjects (Table 3).

Up to present, epidemiologic examination of patients suspected to have monkey pox was carried out only when the diagnosis had been confirmed by laboratory tests. This implies that the material taken from a patient had to take a long way: from a far-away village in the jungle to a district centre, then to Kinshasa, to WHO Headquarters in Geneva and finally, to one of the WHO Collaboration Centers in Moscow or Atlanta. It naturally required a special organization and much effort not to mention the inevitable delay and financial expenses. Therefore, it seemed worthwhile to develop sensitive tests that could be used in the field to detect rapidly orthopox virus antigen. Two methods have been proposed: ELISA with an immunosorbent prepared in advance (IgG isolated from hyperimmune serum to vaccinia virus and sorbed on polystyrene plates) and PHA with immunoglobulin sheep RBC diagnosticum (Shelukhina *el al.*, 1983). The sensitivity of the methods was assessed as compared with the isolation of the virus on chick embryo CAM from the material taken from patients suspected to have monkey pox. The material was stored in a diluted condition for 1 to 9 months after the virus isolation at 4—6 °C.

The tests have demonstrated that both methods were highly sensitive. Although the samples were stored over a long time the orthopox virus was detected by these methods at a slightly lower rate than that ensured by isolation on CAM (23.0 % for PHA, 22.7 % for ELISA and 37.4 % for isolation on chick embryo CAM). No false positive results were obtained by either of the methods.

Discussion

The observations over 17 years (since the disease was discovered) have shown that the current form of monkey pox in humans is of no danger for

the public health. This was the conclusion of the WHO Committee for Orthopoxvirus infection (WHO, 1984). We do not believe, however, that this conclusion means that the infection clinically so similar to smallpox can be dismissed from the specialists' concern as soon as the WHO project has been completed. Therefore, the analysis of the experience gained during surveillance of monkey pox may turn out useful for the organization of a nation-wide control over this infection or for its integration with other WHO projects.

As shown in the present paper monkey pox was confirmed in about 1/3 of suspicious patients. This means that obligatory laboratory analysis that was previously required to confirm the diagnosis was well justified for it saved one the trouble of epidemiological analysis unnecessary in 2/3 of cases. At the same time, this system had an obvious drawback: it was very time-consuming because of the delivery of materials from remote regions of Equatorial Africa to the WHO Collaboration Centers. This not only ensured considerable financial expenses but also caused a delay between verification of diagnosis and seeing the patient. The latter, in its turn, resulted in incomplete and inaccurate epidemiological data.

We believe that the adoption of field diagnostic tests that could be employed for rapid detection of orthopoxvirus antigen in the patients' material would solve the problem.

The data obtained allow us to recommend ELISA and PHA; they were demonstrated to be almost as sensitive as the technique of isolation on chick cmbryo CAM. PHA with stable RBC diagnosticum seems especially attractive, for it is simple and practicable for field conditions and provides an answer on the same working day. Despite of all advantages PHA does not allow to differentiate between different orthopox viruses; however this does not seem very important in the given situation: over 17 years when the authors were carrying out the laboratory tests using samples from Zaire and CAR no other orthopox virus except monkey pox has ever been isolated from any of patients. On the other hand, the utilization of simple and rapid diagnostic method in the field will provide timely and purposeful epidemiological examinations and increase the efficiency of surveillance. At the same time, the control of the properties of monkey pox virus isolated from humans should remain the function of the WHO Collaboration Centers.

Serologic diagnosis of monkey pox deserves special attention. Unlike the above-mentioned methods of orthopox virus antigen detection, serologic diagnosis requires a species identification of antibodies, for considerable proportion of the population still have the antibodies to vaccinia virus as a result of past smallpox vaccinations. The employment of ELISA-A for differentiation of antibodies has demonstrated that it ensures a rapid verification of the monkeypox diagnosis and also provides valuable information about this new human infection (e.g. it allows one to reveal its inapparent forms, to detect the transmission from human to human, etc.). In some cases, however, it was impossible to identify the species of antibodies. RIA-A appeared to be of no use for this purpose, either (Nakano, personal com-

munication). The cause of this failure is not quite clear. The experimental studies and the analysis of diagnostic materials, however, allowed us to suggest two factors that are probably responsible for the appearance of unidentifiable antibodies: early intervals post-infection (week 1 or 2) and consecutive contact with 2 orthopox viruses (in particular, vaccinia virus and monkey pox). On the basis of the experience accumulated so far an additional diagnostic criterion can be proposed for unidentifiable antibodies: the monkey pox diagnosis can be regarded to be reliable if the antibody titre with monkey pox virus is higher than with vaccinia virus. At the same time, difficulties involved with interpretation of results in other cases (with unidentifiable antibodies and equal titres with vaccinia virus and monkey pox virus and also when monkey pox virus is isolated with the titre of antibodies to vaccinia virus being high) call for further improvement of differentiation methods of antibodies to closely related orthopoxviruses. Development of competitive ELISA technique with monoclonal antibodies seems to have good prospects.

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