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Serological Evidence of Infection by Pichinde Virus Among Laboratory Workers

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A total of 44 laboratory workers were examined for serological evidence of infection by Pichinde virus. Complement-fixing antibodies were detected in the sera of 6 of 13 (46%) persons working with the virus but not among 31 persons not working with the virus. Serological evidence of infection was greatest among those workers exposed to concentrated virus. No distinct illness could be associated with the infection.

Certain members of the arenavirus group (14) represent zoonotic hazards to humans. The reservoirs of these viruses are rodents, which are not infrequently persistently infected. Lymphocytic choriomeningitis virus (LCMV) infects mice, and humans may be infected by virus excreted into the domestic environment by the mice (1). A flu-like illness sometimes complicated by meningitis may develop in persons infected by the virus (2, 4, 13, 15, 17). Hemorrhagic fever develops in persons who become infected with either Junin or Machupo viruses, and the source of infection is generally traceable to persistently infected *Calomys* species (6-9). Lassa fever virus also produces illness in humans, and the mortality rate associated with illness produced by this virus is high (3, 5, 10, 20). The other arenaviruses of the Tacaribe complex have not been shown to infect man. One method of determining whether or not a virus can infect humans is to monitor laboratory workers. In the present study, serological evidence of infection by Pichinde virus was sought among persons examining the biological and biochemical nature of the virus.

MATERIALS AND METHODS

Study population. A total of 44 persons from the Departments of Virology and Epidemiology and of Microbiology, Baylor College of Medicine, were studied. Two or more serum samples were analyzed from 35 persons, and single serum samples were analyzed from 9 persons. The persons were categorized into arbitrary risk groups. Individuals of high risk were those persons working with Pichinde virus, whereas individuals of moderate risk were those persons working in the same laboratory area but not with the virus; low-risk individuals were those working with viruses other than Pichinde virus in different laboratory areas of the same building. The persons of the study population were not exposed to other members of the arenavirus group which share cross-reacting antigens. Sera were collected between March 1972 and November 1973. The single serum samples were collected in October 1973. All sera were coded prior to testing, and the code was not broken until results were recorded.

Antibody assay. Blood samples were obtained by venipuncture, the serum was removed from the clotted blood, and all sera were stored at −35 C. Prior to testing, sera were heat inactivated at 56 C for 30 min. Antigen for the complement fixation (CF) test was prepared from infected BHK cell monolayers grown in 16- or 32-oz (0.473- or 0.946-liter) flat-sided prescription bottles. Monolayers were infected with Pichinde virus as previously described (11), and cells were harvested 72 h after infection. Cells were washed three times with borate-buffered saline (BBS), pH 8.2, and then were resuspended at 10% vol/vol in BBS. The resulting cell suspension was frozen and thawed twice, sonically treated for 2 min at 60 kc in a Rayethon sonicator, and clarified by centrifugation at 12,000 × *g* for 30 min at 4 C. The supernatant was stored in portions at −70 C. Control cell antigen was prepared from mock-infected BHK-21 monolayers in the same manner. Antigen preparations were tested with known anti-Pichinde hamster serum prior to use and adjusted to contain 8 U of antigen in the CF test (1:16 dilution). Micro-complement fixation tests were carried out by the method detailed elsewhere (19), using 5 full units of guinea pig complement. All dilutions were made in Veronal-buffered saline containing 0.1% gelatin.

RESULTS

Among persons working with Pichinde virus, 6 of 13 (46%) were found to have antibodies to Pichinde virus (Table 1). No antibodies were found among 14 persons working in the same laboratory area where studies on the virus were being carried out but who were not working with the virus. Antibodies were not detected among

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any of the persons working in other areas of the building.

Biochemical studies were performed by six workers and during the course of these studies large amounts of purified virus were used. Antibodies were found among five of these six persons. Biological studies that entailed primarily preparation of virus stocks, virus assays, and infection of animals were carried out by seven workers, and only one of these persons developed antibodies. This infection was putatively traced to inadvertent self-inoculation that occurred while injecting young hamsters.

The antibody titers in relation to the time when the persons began working with the virus are shown in Fig. 1. A fourfold rise in antibody titers is evident with two persons, F.F. and M.B., and a fourfold fall in titers is evident with two persons, G.H. and B.R. Serious illness was not associated with any of the infections, although during the period of study minor febrile illnesses were experienced. Because others in the laboratory also experienced minor febrile illness, it is not possible to attribute any specific illness pattern to Pichinde virus infection.

A sixth worker (W.R.), not shown in the figure, concentrated Pichinde virus on 24 October 1973, and no CF antibodies were found in a serum sample obtained 30 October 1973. Slight dysuria, myalgia, and a pruritic vesicular eruption of the dorsum of the left thumb developed 22 November 1973. The vesicles became hemorrhagic on the third day of the illness and then resolved. Serum obtained 28 November 1973 had a CF titer of 1:16.

TABLE 1. *Antibodies to Pichinde virus among laboratory personnel with different exposures*

Risk group	No. tested	CF antibodies to Pichinde virus	
		No. positive	Positive (%)
High	13	6	46
Moderate	14	0	0
Low	17[a]	0	0

[a] Paired serum samples on eight persons and single serum samples on nine persons.

DISCUSSION

LCMV was found very early to be hazardous to laboratory workers. Numerous cases of nonfatal and fatal LCMV disease have been reported in the literature. Baum et al. described 10 cases of influenza-like illness in workers exposed to LCMV-infected hamsters (2). Scheid et al. reported a case of LCMV infection with meningitis in a laboratory worker (15), and Smadel et al. described two fatal cases of laboratory-acquired LCMV disease (17). A common factor to all of these cases was exposure to LCMV-infected animals. This mimics the natural situation in which LCMV is acquired by contact with chronically infected gray mice, *Mus musculus*, or their excreta (1, 4). There is no evidence to suggest that LCMV disease is readily spread from person to person.

Lassa fever virus has been reported to have caused a number of fatal and nonfatal infections in hospital and laboratory personnel (5, 10, 20). In the case of Lassa fever, the virus appears to be more readily spread from person to person via the saliva, urine, and other body

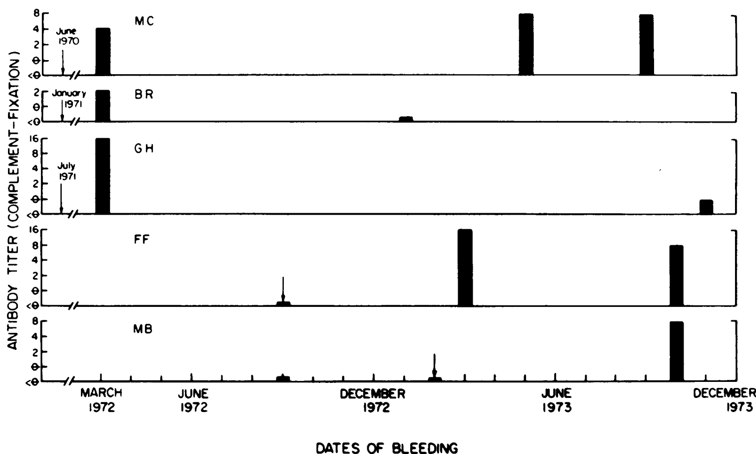

FIG. 1. *CF antibody titers to Pichinde virus in relation to time of possible exposure. Arrows indicate the time when the individuals began working with Pichinde virus.*

fluids (3). Symptoms of Lassa fever are severe, and the case fatality rate is in the range of 40 to 50% (20). All of the reported outbreaks of Lassa fever have occurred in a hospital environment, and in each instance the source of infection of the index case was unknown (5, 20). One case of accidental laboratory infection with Lassa fever has been reported (3). The individual contracted the disease while working with Lassa virus-infected cell cultures and mice.

The hazardous nature of Machupo virus has been emphasized by Peters and co-workers (12). Based on epidemiologic observations, Machupo virus, like LCMV, apparently has a low propensity for person-to-person spread (6). In the natural situation, humans are infected by exposure to persistently infected *Calomys callosus* (7, 9).

Pichinde virus persistently infects *Oryzomys albigularis* but has not been shown to cause naturally occurring human disease (8, 18). The results of this study indicate that, in the laboratory, exposure to high concentrations of Pichinde virus can result in infection of humans. Apparently this capability is dose related, as those exposed to known high concentrations of virus showed the highest percentage of seroconversion. Pichinde virus does not appear to be highly contagious because there was a lack of evidence of infection among those in the moderate risk group.

No definite illness could be associated with the Pichinde virus infection. In one worker where the infection could be traced to a 1-month period, mild dysuria, myalgia, and a localized vesicular eruption of one hand, which could represent the portal of entry, were the only symptoms noticed. In the future it will be necessary to carefully monitor those individuals working with Pichinde virus in order to critically assess the nature of the illness, if any, caused by this agent. The results of this study point out the need to observe safety precautions whether working with known pathogenic viruses, or putative nonpathogens, and to carefully monitor laboratory personnel working with such agents.

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