COMPARATIVE PATHOLOGY OF NORTH AMERICAN AND CENTRAL AFRICAN STRAINS OF MONKEYPOX VIRUS IN A GROUND SQUIRREL MODEL OF THE DISEASE

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Abstract. The first human cases of monkeypox (MPX) were recognized in central Africa in 1970. Since then, sporadic outbreaks of the disease have occurred in central and west Africa. In 2003, an outbreak of human MPX occurred in the United States after importation of infected rodents from west Africa. Clinical features of the 2003 outbreak were less severe than accounts of the disease among people in central Africa. The reasons for this observed difference are unknown. In this study, the clinical and pathologic characteristics of experimental infection with representative central African and North American MPX virus strains were compared in a ground squirrel model of the disease. The results indicate that the US 2003 virus, which phylogenetically is a member of the west African MPX virus clade, was less virulent than central African MPX virus strains.

INTRODUCTION

Monkeypox (MPX) was first identified as a human pathogen in 1970 in Zaire, now the Democratic Republic of the Congo.1 Since then, sporadic human MPX outbreaks have occurred in forested regions of central and west Africa, with most cases occurring in the Congo River Basin.2 The clinical presentation of this disease in humans resembles smallpox, and human cases of MPX in central Africa have been reported to be more severe than those in west Africa.3–5 Morbidity and mortality of the disease have been highly variable in African outbreaks, with case fatality rates ranging from 1% to 20% and a significant secondary transmission rate.6–8 When human MPX occurred in North America, it appeared to have a very different clinical course than in Africa.9–11 Although the North American and central African outbreaks were caused by genetically different strains of MPX virus,12 it is unclear whether the observed differences in clinical presentation were caused by virulence differences among MPX strains or by other host or environmental factors.

Based on descriptions of several outbreaks that occurred in central Africa between the 1970s and the 1990s, the clinical presentation of monkeypox cases in that region has been similar to human smallpox of the ordinary type.5–7 Onset of symptoms began with fever, malaise and lymphadenopathy, followed by the development of a rash that passed through the classic poxvirus stages: macular, papular, vesicular, and pustular, before umbilication and detachment.6 A hemorrhagic type rash, as seen in severe smallpox, was not usually observed, but a confluent rash occurred in almost 10% of African MPX patients and was strongly associated with severity of disease.5 A recurrent febrile period was indicative of severe disease and of probable fatal outcome.2 Upper respiratory tract involvement (tonsillitis, pharyngitis) was often observed during the febrile stage, and coagulation disorders, respiratory distress, and multi-organ failure occurred at a later stage in severe cases.2

No human MPX outbreak had occurred outside of the African continent until 2003, when imported African rodents introduced the virus into the United States. This introduction caused an outbreak that resulted in 37 confirmed human cases.13,14 The clinical spectrum of disease observed in the North American (NA) MPX outbreak was different from outbreaks previously observed in central Africa. Most of the NA cases had a mild flu-like onset, a small number of lesions, and self-limited disease.10,14 Only two pediatric cases developed a more severe disease that required intensive care. All infected individuals recovered in the North American 2003 outbreak; furthermore, the number of lesions per case was lower compared with African cases, and no secondary transmission was observed.14,15

Likos and others12 and Chen and others16 recently compared the genomic and proteomic characteristics of the MPX virus responsible for the 2003 outbreak with representative African strains of the virus and found similarities between the NA virus and several west African MPX virus isolates. Monkeypox outbreaks in west Africa have also been characterized by lower mortality and secondary transmission rates than central African outbreaks12,16 but the explanation for these observed differences is unknown.

Another method for comparing virulence among different genetic or geographic virus isolates is to observe differences in their clinical presentation, mortality, and pathology in animal models. We recently reported a ground squirrel model of severe MPX virus infection, which was originally developed to test experimental therapeutics for the treatment of smallpox and other orthopoxvirus infections.17,18 Using the ground squirrel model, we compared the pathogenicity of a central Africa and a North American isolate of MPX virus. This paper reports the results of that study.

MATERIALS AND METHODS

Study design. Comparative pathology of MPX US03 and MPX Z79 viruses. Forty ground squirrels were used in this study, 20 in the US03 group, and 20 in the Z79 group. Animals in the two different groups were matched based on weights. A group of five additional uninfected animals was used to obtain control values on the blood tests. All infected animals received 100 plaque-forming units (PFU) of the respective virus strain that was injected subcutaneously (sc) in the right thigh. On days 2, 4, 6, 8, and 10 after infection, blood samples
were collected by cardiac puncture under anesthesia from eight animals per group; three of these animals, randomly selected, were subsequently killed and dissected. On day 12 post-infection (pi), the experiment was terminated and any remaining survivors were killed.

Statistical analysis. Survival data were analyzed using the Kaplan-Meier method. The biochemical and hematologic results were analyzed with one-way analysis of variance. Statistical significance was accepted for $P \leq 0.05$. Data were expressed as the mean ± SD.

Viruses. Two different strains of MPX virus were used in the study. The first, designated MPX Z79, was isolated in 1979 from a fatal human case in Zaire. The second, designated MPX US03, was isolated from a non-fatal human case in the United States in 2003. Both virus strains were kindly provided by Dr. Inger Damon (Centers for Diseases Control and Prevention, Atlanta, GA). Two MPX virus stocks were prepared in Vero 76 cells and were adjusted to the following titers: 1.8 × 10^7 PFU/mL for the US03 MPX strain and 3.7 × 10^6 PFU/mL for the Z79 MPX strain. Virus inocula of 100 PFU/mL were prepared in phosphate-buffered saline, pH 7.4, containing 10% fetal bovine serum (diluent) from both stocks, to be used in the comparative study.

Animals. Animals used in the study were wild-caught 13-lined ground squirrels (Spermophilus tridecemlineatus) obtained from TLS Research (Bloomingdale, IL). Animals were cared for in accordance with guidelines of the Committee on Care and Use of Laboratory Animal Resources, National Research Council, under an animal use protocol approved by the University of Texas Medical Branch. All studies with infected animals and their tissues were carried out by persons recently immunized with the Dryvax® smallpox vaccine (Wyeth Laboratories, Marietta, PA) who were positive-pressure high efficiency particulate absorbing (HEPA)-filtered full-face respirators and worked within biosafety level 3 select agent-approved facilities. To obtain samples for virus studies, clinical laboratory tests, and histologic analysis, three animals from each group were killed on days 2, 4, 6, 8, and 10 pi.

Virus assay. Samples for virus assay (blood, liver, spleen and lung) were triturated individually in sterile 7-mL glass TenBroeck tissue grinders (Kimble/Kontes, Vineland, NJ) in 3.0 mL of diluent, centrifuged, diluted, and inoculated into microplate cultures of Vero cells using a double agar overlay as described. The MPX virus plaques were read on the sixth day, and titers were calculated as PFU/mL of blood or a 10% (v/v) organ homogenate.

Clinical laboratory values. Hematologic assays were performed directly on EDTA-treated whole blood using a Hemavet 950 analyzer (Drew Scientific, Oxford, CT), which determined total white blood cells, differential counts, neutrophils, monocytes, lymphocytes, basophils, eosinophils, red blood cells, hemoglobin, hematocrit, and platelets.

For clinical chemistry studies, whole blood collected in plain glass tubes was allowed to clot at room temperature for several hours and then centrifuged for 5 minutes at 2,500 rpm. Serum was transferred to clean tubes and analyzed promptly on a Prochem-V clinical chemistry analyzer (Drew Scientific), according to the manufacturer’s instructions. The following biochemical parameters were determined: serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), total bilirubin, alkaline phosphatase, serum amylase, blood urea nitrogen, and serum potassium.

For coagulation studies, citrated blood was centrifuged at 2,500 rpm for 10 minutes at 4°C. After centrifugation, the plasma was transferred to clean tubes and analyzed on a Star-14 coagulation analyzer (Diagnostica Stago, Parsippany, NJ), and the following values were determined: prothrombin time (PT), activated partial thromboplastin time (aPTT), fibrinogen, thrombin time (TT), factor X (deficient), factor VIIa (VIIa), and protein C.

Histopathologic and immunohistochemical studies. After exsanguination, a necropsy was performed on each animal and samples of the following tissues were collected: liver, spleen, pancreas, kidney, adrenal gland, lung, heart, lymph nodes, and intestine. Chunks of the above tissues were fixed in 10% neutral-buffered formalin for 36 hours and then were transferred to 70% ethanol before being processed for routine paraffin embedding. Several 4-5-μm sections were made from each tissue, and two were stained by the hematoxylin and eosin method. Other unstained sections were used for immunohistochemical studies to localize viral antigen. A vaccinia mouse hyperimmune ascitic fluid was used as the primary antibody and was conjugated with streptavidin-peroxidase as described.

Semiqualitative immunohistochemistry was performed by counting the number of positively stained cells per high-power field (20× and 40×) on three different sections, and averaging five fields for each section analyzed, for a total of 15 fields per animal and 45 per time point. The value for a single time point was obtained as an average of all animals killed on that particular day pi analyzing three sections per animal.

RESULTS

Clinical presentation, morbidity, and mortality. During the first two days after infection with MPX virus, the animals in both infected groups (MPX US03 and MPX Z79) appeared grossly normal. On day 3 pi, most animals infected with the Z79 virus strain were anorexic and appeared lethargic, but the same symptoms did not appear until 1–2 days later in the animals infected with MPX US03 virus. Nosebleeds were frequently seen in animals from the Z79 group after day 4 pi, but were seldom observed in the US03 group. From day 5 pi, most animals infected with the MPX Z79 virus strain had respiratory distress, apparent labored breathing, and audible wheezing; in the US03 group, these same symptoms were observed only as a terminal event, within 24 hours prior to death. Both stains of MPX virus were 100% lethal at the dose used (100 PFU), but the time to death was different: all deaths occurred between days 6 and 11 in the Z79 group and between days 7 and 11 in the US03 group.

Viral load. Table 1 shows the mean virus titers at various days pi in blood, lung, liver, and spleen of ground squirrels infected with the two MPX virus strains. The viral load increased until death in all samples analyzed. Animals infected with the Z79 MPX virus strain showed consistently higher virus titers in blood and lung tissue, compared with those infected with the US03 virus isolate.

In previous studies, we had calculated the 50% lethal dose ($LD_{50}$) for sc infection with the two MPX virus strains in ground squirrels, using the method of Reed and Muench, and obtained $LD_{50}$ values of 0.46 PFU for the US03 isolate and 0.35 PFU for the Z79 isolate (Sbrana E, 2006. Pathology and Experimental Therapy of Severe Orthopoxvirus Infection.)
Clinical laboratory findings. White blood cell counts were elevated in the infected animals, regardless of the MPX virus strain used (Figure 1). There was no significant difference between the values obtained in animals inoculated with either the Z79 or US03 virus strains.

The values of ALT, AST, total bilirubin, and alkaline phosphatase were also elevated in the infected animals compared with normal controls; but again no statistical difference was observed between these clinical chemistry values in the two infected groups (Z79 or US03) (Figure 2).

Figure 3 shows the mean coagulation values on day 7 pi for three ground squirrels infected sc with the two MPX strains. The PT, aPTT, and TT were elevated in all of the infected animals, compared with normal controls; but again no statistical difference was observed between these clinical chemistry values in the two infected groups (Z79 or US03) (Figure 2).

Figure 3 shows the mean coagulation values on day 7 pi for three ground squirrels infected sc with the two MPX strains. The PT, aPTT, and TT were elevated in all of the infected animals, compared with normal controls; but again no statistical difference was observed between these clinical chemistry values in the two infected groups (Z79 or US03) (Figure 2).

Histopathologic and immunohistochemical studies. US03 group. The first noticeable histopathologic changes in the MPX-infected squirrels occurred in the lymphoid organs and in the respiratory system. Animals infected with the MPX US03 virus showed neutrophils recruited into the respiratory epithelium (both in the trachea and the bronchi) from day 3 pi onward (Figure 4A). When stained for the presence of MPX viral antigen, these areas showed positive staining (Figure 4B). More neutrophils were also observed in the lung from day 4 pi onward (Figure 4C).

A few apoptotic lymphocytes and phagocytes could be recognized in the peritracheal lymph nodes after day 2 pi. Positive immunostaining was seen after day 2 pi in the thymus that involved the medulla and the area surrounding the interlobu-

<p>| Table 1 | Mean virus titers in blood, lung, liver, and spleen of ground squirrels infected subcutaneously with 100 plaque-forming units (pfu) of MPX virus strains US03 and Z79* |</p>
<table>
<thead>
<tr>
<th>Tissue sample</th>
<th>Virus strain</th>
<th>D-0</th>
<th>D-2</th>
<th>D-4</th>
<th>D-6</th>
<th>D-8</th>
<th>D-10</th>
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<tr>
<td>Blood</td>
<td>US03</td>
<td>0†</td>
<td>0</td>
<td>2.6 ± 0.1</td>
<td>4.4 ± 0.2</td>
<td>4.8 ± 0.2</td>
<td>5.3 ± 0.4</td>
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<tr>
<td></td>
<td>Z79</td>
<td>0</td>
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<td>3 ± 0.1</td>
<td>4.5 ± 0.3</td>
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<td>6.1 ± 0.3</td>
</tr>
<tr>
<td>Liver</td>
<td>US03</td>
<td>0</td>
<td>0</td>
<td>2.6 ± 0.1</td>
<td>5.2 ± 0.3</td>
<td>7.3 ± 0.2</td>
<td>8.4 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>Z79</td>
<td>0</td>
<td>0</td>
<td>3.3 ± 0.1</td>
<td>5.5 ± 0.1</td>
<td>7.1 ± 0.4</td>
<td>7.9 ± 0.2</td>
</tr>
<tr>
<td>Spleen</td>
<td>US03</td>
<td>0</td>
<td>0.4 ± 0.1</td>
<td>3.1 ± 0.2</td>
<td>5 ± 0.2</td>
<td>6.5 ± 0.1</td>
<td>7.5 ± 0.2</td>
</tr>
<tr>
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<td>0</td>
<td>1.1 ± 0.2</td>
<td>3.5 ± 0.3</td>
<td>5.8 ± 0.2</td>
<td>6.9 ± 0.2</td>
<td>7.6 ± 0.1</td>
</tr>
<tr>
<td>Lung</td>
<td>US03</td>
<td>0</td>
<td>0</td>
<td>2.4 ± 0.2</td>
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<td>6.8 ± 0.2</td>
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<tr>
<td></td>
<td>Z79</td>
<td>0</td>
<td>1.6 ± 0.3</td>
<td>3.7 ± 0.2</td>
<td>6.1 ± 0.1</td>
<td>7.7 ± 0.1</td>
<td>8.9 ± 0.1</td>
</tr>
</tbody>
</table>

* Results are mean ± SD (n = 3 animals per group).
† Virus load is the log$_{10}$ pfu/mL of blood or/mL of a 10% organ homogenate. 0 = < 0.7 pfu/mL.

Figure 1. Mean white blood cell counts for ground squirrels infected with monkeypox virus strains US03 and Z79 and uninfected controls. Values were determined on day 7 post-infection (n = 8 per group). WBC = white blood cells; NE = neutrophils; LY = lymphocytes; MO = monocytes; EO = eosinophils; BA = basophils. Error bars show the standard deviation.
lar septa, and in lymph nodes, mainly in the medulla, paracortex, and around the trabeculae (Figure 4D). A few antigen-presenting cells (probably follicular dendritic cells) showed very intense positive staining (Figure 4D, inset).

Later in the infection at days 7–8 pi, edema and hemorrhage were evident in the lung, as well as more macrophages and fibroblasts (Figure 4E). At this point in time, limited viral antigen staining was observed in the lung parenchyma (Figure 4F), but immunostaining was intense in the vascular endothelium and the connective tissue of the lamina propria (Figure 4G), the bronchial epithelium (Figure 4H), and the mucosal-associated lymphoid tissue (MALT) (Figure 4I).

The major pathologic changes in the liver of the infected animals were portal inflammation and midzonal necrosis (Figure 5A), which was observed after day 6 pi in most cases. Viral antigen staining initially involved scattered groups of necroapoptotic hepatocytes on days 4–5 pi (Figure 5C), but by days 8–9 pi, it had became diffuse. Many cytoplasmic inclusion bodies were visible at this point in the hepatocytes, and showed intense immunostaining (Figure 5E).

In the spleen, fibrinoid necrosis of the mantle and marginal zone was first observed on day 4 pi and became severe after day 7 pi (Figure 5B). Viral antigen staining was mostly limited to macrophages early in the infection (Figure 5D), but from...
day 8 pi onward most of the cells in the white pulp and what was left of the mantle zone showed positive immunostaining (Figure 5F).

Z79 group. From days 2–3 pi, neutrophilic infiltrates in the respiratory epithelium and pavementing of the pulmonary vascular endothelium were evident in ground squirrels infected with the MPX Z79 isolate (Figure 6A). Karyorrhexis was observed in the lymphoid tissues beginning from days 2–3 pi. As early as day 2 pi, immunostaining showed intense viral antigen staining in the lymph nodes, the thymus, the bronchial epithelium, and the mucosal-associated lymphoid tissue (Figure 6B and C). Several alveolar macrophages, as well as pneumocytes (mainly type II) showed positive immunostaining from day 3 pi onward (Figure 6D).
After day 6 pi, severe pulmonary edema and hemorrhage were observed in the lungs of most of the infected animals (Figure 6E). Many swollen macrophages were seen in the lungs, as well as several multinucleated histiocytes (Figure 6E inset). On days 5–6 pi, viral antigen was seen in the lung involving several cells per high-power field, mainly alveolar macrophages, pneumocytes, and vascular endothelial cells (Figure 6F and inset).

Starting on day 3 pi, more neutrophils were also seen in the liver sinusoids around the terminal hepatic venules, and inflammatory infiltrates composed mainly of monocytes were observed around the portal tract. Later in the infection, after day 4 pi, several abscesses were seen in the livers of the infected animals (Figure 7A), and many dying hepatocytes could be recognized (Figure 7A, insets). At this point in the infection, numerous hepatocytes showed cytoplasmic inclusion bodies that stained positive for viral antigen both in the centrilobular (Figure 7C) and portal areas (Figure 7E).

In the spleen, lymphoid necrosis became apparent on day 3 pi, and from day 6 pi onward fibrinoid necrosis and apoptosis...
of lymphocytes were seen in most animals (Figure 7B and inset). Viral antigen was also demonstrated by immunostaining in the white pulp of the spleen and in the surrounding mantle and marginal zones, the perilymphoid red pulp (Figure 7D), and on the capsule (Figure 7F).

The proportion of cells that stained positive for MPX viral antigen in the various tissues were recorded for all animals killed, and a comparison between the US03 and the Z79 groups was made. The average numbers of positive cells per high-power field are shown in Table 2. The number of cells positive for viral antigen in sections of lung and spleen were consistently higher for the animals infected with the Z79 virus strain at each point after infection. On days 8 and 10 pi, the number of positive cells in the liver sections was also higher for the animals infected with the Z79 virus.

**DISCUSSION**

The two MPX virus isolates used in this study, US03 and Z79, both produced a fulminant disease in the ground squir-
rels, with characteristics that were similar to those described in cases of human hemorrhagic smallpox. Both MPX virus strains were 100% lethal by the sc route at doses higher than 1 PFU. From careful observation of the infected animals, the onset of severe respiratory distress appeared more uniform and rapid in the MPX Z79 group. Bleeding diatheses were also evident in this group after day 4 pi, and they were infrequently observed in the US03 group. In general, animals infected with the MPX Z79 virus strain also began to die earlier.

Although differences were observed in the severity of the clinical presentation and histopathology among the two groups, there was little difference between the LD<sub>50</sub> values for the two virus strains (0.35 PFU for MPX Z79 and 0.46 PFU for MPX US03). However, there was a statistically significant difference in the virus titers in the blood and lung, which indicated that animals infected with the central African MPX virus isolate developed a higher viremia and a higher viral load in their lungs during the course of the infection. This result was consistent with our observations in intraperitoneally and intranasally infected ground squirrels, namely

**Figure 7.** Selected micrographs of tissue sections of ground squirrels infected with monkeypox virus strain Z79. A, day 5 post-infection (pi), liver (hematoxylin and cosin stain, original magnification × 10) ([Insets, × 40 [top], × 100 [bottom]]). B, day 7 pi, spleen (hematoxylin and cosin stain, original magnification × 20) ([Inset × 100]). C, day 6 pi, liver (immunoperoxidase stain, original magnification × 20). D, day 6 pi, spleen (immunoperoxidase stain, original magnification × 20). E, day 6 pi, liver (immunoperoxidase stain, original magnification × 40). F, day 8 pi, spleen capsule (immunoperoxidase stain, original magnification ×40).
that the viral load in the lung and blood after intraperitoneal and intranasal inoculation was 1–2.5 logs higher in the MPX Z79 virus–infected animals compared with the MPX US03 virus–infected animals. The higher viral load and intensity of viral antigen observed in the respiratory tract of the squirrels infected with the MPX Z79 virus strain might explain the higher rate of secondary transmission (suspected to occur in part by the aerosol route) reported during human outbreaks in the Congo River Basin.2

Both groups of squirrels showed increased white blood cell counts and elevated liver transaminase levels during the infection, but the differences between the two groups were not statistically significant.

The coagulation parameters also appeared more severely impaired in the animals infected with the MPX Z79 virus isolate compared with those infected with the US03 virus. Although the values of PT, aPTT, and TT were elevated for both groups compared with uninfected controls, the values for animals in the Z79 group were approximately 50% higher than those in animals of the US03 group. The depletion of fibrinogen and factor X was also more severe in the animals infected with Z79 strain. Animals from the Z79 group showed a much longer clotting time when probed with factor X-deficient plasma. Protein C levels were lower but still detectable in the US03 group compared with the controls. In contrast, protein C levels were undetectable in the animals infected with Z79 strain. Animals from the Z79 group showed a much longer clotting time when probed with factor X-deficient plasma. Protein C levels were lower but still detectable in the US03 group compared with the controls. In contrast, protein C levels were undetectable in the animals infected with Z79 strain. Factor VIIa was approximately three-fold higher in the animals infected with MPX Z79 group compared with the controls and the animals infected with MPX US03 virus.

The elevated level of fVIIa and the numerous neutrophils seen in tissue sections of the MPX virus–infected animals suggest that the coagulation disorders could be due to extravasating neutrophils causing tissue damage and fluid imbalance. This may indicate that the depletion of clotting factors is caused by excessive activation of the extrinsic (tissue factor–dependent) pathway, a theory that is consistent with a presumptive diagnosis of consumptive coagulopathy (disseminated intravascular coagulation). These histopathologic and laboratory results also support the clinical observation that many of the animals infected with the MPX Z79 virus isolate showed symptoms of bleeding disorders during the course of the disease.

The pathologic changes observed in tissue sections of infected animals in the two groups were similar and were consistent with those reported previously by our group.17,18 The main histopathologic changes were seen in the liver (hepato-cellular necrosis and portal inflammation), lymphoid tissue (lymphocytic apoptosis and fibrinoid necrosis of the mantle and marginal zone and of the perilymphoid red pulp), and respiratory tract (pulmonary edema and hemorrhage). Tissue damage in the lung appeared more severe for the animals infected with the Z79 central African isolate of MPX virus. The appearance of pulmonary edema and hemorrhage occurred earlier in the course of infection in the Z79 group compared with the US03 group, and numerous multinucleated giant cells were seen in lung sections of the MPX Z79 virus–infected animals.

The MPX viral antigen was detected by immunohistochemical analysis in the lung, liver, lymphoid organs, esophagus, and bowel. In general, the intensity and diffusion of viral antigen staining in the respiratory tract was higher in the animals infected with the Z79 isolate compared with those infected with the US03 isolate. For instance, squirrels infected with MPX Z79 showed numerous antigen-positive cells in the lung parenchyma early after infection, approximately fourfold higher than in the US03 group. The intensity of the immunostaining was also much higher in the Z79 group compared with the US03 group. These observations might suggest more efficient viral replication and cell-to-cell spread of the central African MPX isolate. More efficient replication and viral spread is consistent with the finding of higher viremia and with the higher number of animals with pox-like lesions in the Z79 group. Interestingly, human MPX cases in central Africa also have been reported to show a higher number of skin lesions than cases observed in west Africa or in North America.9–11,14,15 Since epidemiologic and phylogenetic evidence suggests that the 2003 North American MPX outbreak was caused by the introduction of a west African strain of the virus,12 we have assumed that the US03 isolate is representative of west African MPX viruses.

Based on that assumption, the higher virus titers in blood and in the respiratory tract, more severe pulmonary pathology, and impaired coagulation functions found in the Z79 group compared with the US03 group suggest that central African MPX virus strains are more virulent than their west African counterparts. Although our study only compared one MPX virus strain representative of central and west Africa, the results seem to confirm epidemiologic and clinical observations among human cases in the two regions; namely that MPX in central Africa has a higher morbidity, mortality, and human-to-human transmission rate than the disease in west Africa.12

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### Table 2

**Number of viral antigen–positive cells per high-power field (20×) in tissue sections of lung, liver, and spleen of ground squirrels infected subcutaneously with 100 plaque-forming units of MPX strain US03 or Z79**

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Day 2</th>
<th>Day 4</th>
<th>Day 6</th>
<th>Day 8</th>
<th>Day 10</th>
</tr>
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<tbody>
<tr>
<td>Lung US03</td>
<td>0.0 ± 0</td>
<td>4.0 ± 1</td>
<td>12.5 ± 2</td>
<td>15.5 ± 3</td>
<td>17.0 ± 3</td>
</tr>
<tr>
<td>Z79</td>
<td>10.0 ± 1</td>
<td>42.5 ± 9</td>
<td>69.0 ± 3</td>
<td>84.5 ± 19</td>
<td>83.0 ± 14</td>
</tr>
<tr>
<td>Liver US03</td>
<td>5.5 ± 2</td>
<td>25.0 ± 1</td>
<td>50.5 ± 6</td>
<td>100.0 ± 1</td>
<td>95.5 ± 2</td>
</tr>
<tr>
<td>Z79</td>
<td>2.0 ± 1</td>
<td>13.5 ± 1</td>
<td>47.5 ± 2</td>
<td>145.0 ± 31</td>
<td>151.0 ± 6</td>
</tr>
<tr>
<td>Spleen US03</td>
<td>6.0 ± 3</td>
<td>17.5 ± 2</td>
<td>116.5 ± 18</td>
<td>133.0 ± 11</td>
<td>146.5 ± 11</td>
</tr>
<tr>
<td>Z79</td>
<td>38.0 ± 8</td>
<td>75.0 ± 11</td>
<td>125.0 ± 18</td>
<td>172.0 ± 6</td>
<td>180.0 ± 4</td>
</tr>
</tbody>
</table>

* Three animals were examined per time point in each group. Fifteen fields examined per animal, 5 per section (3 total sections). Numbers are the mean ± SD of 45 fields examined.
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