A case of Monkeypox in a baby monkey

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ABSTRACT

Despite having a low fatality rate in monkeys, monkeypox remains an important disease because of its zoonotic potential. The aim of this study is to describe the clinical, pathological, ultrastructural and virological findings of the first monkeypox case diagnosed in a baby monkey in Türkiye with unknown transmission. A monkeypox infection was detected in a 1-month-old, female, baby monkey born in Antalya Zoo and died with skin lesions. It was reported that the baby’s mother did not care for and caregivers took care of the cub, and skin lesions on the hands of the caregivers and responsible veterinarian were reported. Necropsy, histopathology, electron microscopy and chorioallantoic membrane (CAM) test were performed. Typical cutaneous and pulmonary poxvirus finding in a baby monkey was described in this case. CAM results firstly reported in a monkeypox case. Because of the zoonotic potential of illness, monkey colonies, particularly in zoos, must be controlled with vigilance. This is the first monkeypox report in a baby monkey in Türkiye.

Keywords
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Monkeypox is an important disease because of its zoonotic potential, despite the fact that it has a low mortality rate in monkeys. A virus that produces smallpox-like infection in humans causes the disease and belongs to the orthopoxvirus. The virus is relatively large, having a diameter of 200-250 nanometers with a collapsed center. Although they translate mRNA on host ribosomes, their genomes include all of the required transcription, replication, egress, and assembly proteins (1).

The first natural case of monkeypox in monkeys was reported in 1959 in a cynomolgus macaque colony. It was characterized by typical poxvirus lesions forming on the skin, and no deaths were documented (9). Immediately following that, in 1960, a spontaneous case of monkeypox in both rhesus and cynomolgus macaques was reported (8). Only juvenile death was reported in cynomolgus macaques in that case, and ulcerative mucosal lesions, hemorrhagic skin lesions, facial edema, and generalized lymphadenopathy were described in dead monkeys. The histopathological examination of the skin lesions revealed that they were extremely comparable to human smallpox lesions (2).

The disease is spread through skin sores, body fluids or excretions of affected animals’ respiratory system, and direct or indirect contact with contaminated materials. Although human-to-human transmission is feasible, disease transmission between humans is extremely infrequent (1, 3). Viral isolation or PCR can be used to confirm a conclusive diagnosis of monkeypox infection. Electron microscopy imaging and immunohistochemical staining for orthopoxvirus antigens can also be conducted (7).

The first case of monkeypox in humans was reported in Türkiye on June 30, 2022 and eleven cases of monkeypox have been documented so far. The purpose of this study is to present the clinical, pathological, ultrastructural, and virological findings of the first monkeypox case diagnosed in a baby monkey in Antalya Zoo in 2019. Because monkeypox has recently spread rapidly in humans, the goal is to assess the data and call the attention of those who have contact with animals, particularly veterinarians.
Figure 1. Necropsy findings of the baby monkey died from the monkeypox.

a. Macroscopical appearance of the skin lesions initial hyperemic lesion and crusted papules (arrows).
b. Closer appearance of the lesions (arrows).
c. Histopathological appearance of the skin lesions, thick necrotic crust (arrow) on the epidermis and hyperemic dermal vessels (arrow heads), HE, Scale bar=100µm.
d. Marked hyperemia (arrow heads) and inflammatory cell infiltrations in dermis and intracytoplasmic inclusion bodies (thick arrow) in epidermal cells, HE, Scale bar=100µm.
e. Hyperemia (arrow heads) and inflammation and intracytoplasmic inclusion bodies (thick arrows), HE, Scale bar=100µm.
f. Marked hyperemia (arrow heads) and inflammatory cell infiltration and intracytoplasmic inclusion bodies (thick arrows), HE, Scale bar=100µm.
A female monkey imported from Africa to Antalya Zoo gave birth to a female baby monkey at the end of the regular gestation time. However, skin lesions were reported to emerge within a few weeks. Because the mother did not care, the baby was fed by the caregivers, and then respiratory system issues arose, and the baby died when it was around one month old.

The baby monkey was taken to the Department of Pathology at Burdur Mehmet Akif Ersoy University, Faculty of Veterinary Medicine for the diagnosis of the disease. She was thoroughly examined, and tissue samples were taken from lesioned skin and all internal organs. During necropsy, tissue samples were fixed in 10% neutral formalin solution (Formaldehyde solution, Cat. No: 1.04002, Merck, Missouri, USA) for histopathological evaluation and 2.5% glutaraldehyde solution (Glutardialdehyde solution 25%, Cat. No: 111-30-8, Merck, Missouri, USA) for ultrastructural study. The remaining tissue samples were stored at -20 °C for CAM inoculation.

At necropsy, crusted and papular lesions were more visible, especially on the face, armpit, abdomen, chest, and extremities. The skin was covered with markedly hyperemic plaque-like lesions. Initial skin lesions often grow over the first four days becoming deeply localized, firm, and 5 to 20 mm in size before crusts form (Figure 1a-b). Except for severe pleuropneumonia, no macroscopic findings in visceral organs were noted (Figure 1c). The other organs were not histopathologically examined due to the autolytic changes.

Histopathological examinations revealed marked hyperemia of the cutaneous vessels. The epidermis was densely thickened, and the crusts formed as necrotic masses on the epidermis (Figure 1d). Hydropic degeneration and necrosis were noticed, especially in stratum spinosum cells. Ulcers were also found in several

Figure 2. a. Histopathological appearance of the pneumonic lung, hyperemia, emphysema and marked inflammatory reaction (arrows), HE. b. Typical pockformations (arrows) on the CAM after inoculation. c. Microscopical appearance and intracytoplasmic inclusions (arrows) in the CAM cells. HE. d. Ultrastructural view of the lesions, typical centrally collapsed pox viral particles (arrows) in the epidermal cell cytoplasm, Scale bar= 0.2µm.
areas. Some keratinocytes were found to have inclusion bodies (Figure 1e-f). Inflammatory cell infiltrates were seen, with the dermis showing the most. The skin lesions resembled those seen in human smallpox. Hyperemia of the lungs’ arteries, thickening of the alveolar septal tissue, and inflammatory cell infiltrations were observed. Desquamation of the epithelial cells of the alveoli, bronchioles, and bronchus was also seen (Figure 2a).

Inoculation of an embryonated chicken egg (11-12 day-old) with an air sac to CAM following homogenized skin samples indicated thickening and typical pox lesions (Figure 2b). Histopathological examination of the CAM revealed epithelial cell proliferation and numerous intracytoplasmic inclusion bodies (Figure 2c). Furthermore, lesioned skin and CAM samples were examined ultrastructurally. On electron microscopy, typical pox virus particles were found.

In the cytoplasm of the keratinocytes, naked viroplasm, sequential stages of virus particle development, and mature virions were recognized by transmission electron microscope TEM. Thin electron microscopical sections of skin revealed oval and brick-shaped monkeypox virions within the cytoplasm of epidermal cells. The virions had a typical ovoid to round shape, lateral bodies, and a central core (Figure 2d).

Monkeypox is a viral zoonotic disease that is clinically comparable to smallpox but has milder clinical symptoms. With the end of smallpox vaccination in humans in 1980 due to the smallpox eradication, monkeypox emerged as the most important orthopox virus. The disease is found in Central and West Africa, particularly prevalent in the Democratic Republic of the Congo. Monkeypox has also been found in animals imported from Africa in the United States. Similarly, cases of monkeypox in people have been reported following African-related trips (4). Monkeypox spread fast throughout the world, with countless cases reported from various regions. Although there have only been eleven human cases of monkeypox in Türkiye, no monkeypox virus has been reported in monkeys.

In the experimental model of monkeypox in prairie dogs, the virus multiplies soon after entering the body via the oropharynx, nasopharynx, or intradermal routes, and then spread to the adjacent local lymph nodes. Lymphadenopathy and fever are common signs. The initial lesions appear in the oropharyngeal mucosa, followed by skin eruptions. The rash is accompanied by typical skin lesions, and the disease is characterized by typical papule formation (5). In this case, anamnesis led to the development of skin lesions one week after the onset of the initial clinical signs, suggesting possible pathogenesis in monkeys.

Both virus isolation and serological tests revealed that the most likely viral reservoir is wild squirrels (6). The fact that this was a single case, the mother monkey showed no clinical signs, and the presence of squirrels and other rodents in the environment suggested that these might be reservoirs. The mother, on the other hand, could be subclinically sick or a carrier. Therefore, the source of infection in this case could not be determined.

Dermatitis is the most common and distinguishing feature of the disease in monkeys. It can appear as single small papules at times, but it can also appear as large lesions, mainly in the face, abdomen, inguinal, and thoracic regions. Lesions are less common on the palmar surfaces of the hands and plantar surfaces of the feet. The spread of the lesions increases as the disease progresses. While the early lesions appear on the skin as hyperemic areas, characteristic papules and crusts grow with time (10). In this case, similar typical findings were observed. Within a week, the lesions had spread throughout the body. This rapid spread could be attributed to the monkey’s age. The lesions were mostly localized on the face, abdomen, armpits, and hands. While the lesions initially appeared hyperemic, they eventually took on a typical papular appearance. Crusting and crust shedding were also observed in several areas.

Pneumonia is the leading cause of death in this condition. A common consequence is secondary bacterial septicemia. The lungs become heavy, hard, and dark red in color. The presence of lobular, edematous, and atelectatic regions is common. Although the pleura exhibits an inflammatory reaction, adhesions are rarely found. Hydropericardium may be seen at necropsy (10). In this case, pneumonia was determined to be the cause of death for the animal. Lungs had already described in her findings. Pleuritis occurred, although no adhesions or hydropericardium were found.

In the histopathological examination, epithelial hyperplasia is typical in skin lesions, but necrosis is also seen. Swelling and degeneration of cells in the stratum spinosum are particularly common. It is usual to see intraepithelial vesicopustules with swollen epithelial cells, neutrophils, eosinophilic fluid, and fibrin. It is possible to see intracytoplasmic inclusion bodies in epithelial cells (10). The findings in this case were congruent with those found in the literature. However, necrosis and crusting were more visible, most likely due to the pup's thin skin. Typical inclusion bodies were also frequently encountered.

The respiratory system is the most susceptible system other than the skin. In general, all monkeys who succumb to the disease develop fibrino-necrotic bronchopneumonia. Histopathological findings include diffuse necrotic changes in the bronchial and bronchiolar epithelium, inflammatory cell infiltrations, edema, and fibrin accumulation (10). In this case, the most significant extracutaneous lesion was bronchopneumonia, and the findings were consistent with previous research.
Because of the presence of autolytic findings, the lesions of the other organs were not evaluated. Electron microscopy revealed characteristic collapsed viral particles in both skin lesions and CAM. The animal caregivers and veterinarian caring for the monkey developed typical skin lesions, but these were not examined. The diagnosis of monkeypox in this baby monkey was made based on characteristic macroscopic, histopathological, ultrastructural, and CAM findings.

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The authors declared that there is no conflict of interest.

**Author Contributions**
ÖÖ, MK, VI and HSS conceived and planned the study. ÖÖ and MK carried out the study. ÖÖ, MK, VI and HSS contributed to sample preparation, laboratory examinations and drafting. ÖÖ and MK contributed to the interpretation of the results. ÖÖ and MK took the lead in writing the manuscript. All authors provided critical feedback and helped shape the research, analysis and manuscript.

**Data Availability Statement**
The data supporting this study's findings are available from the corresponding author upon reasonable request.

**Ethical Statement**
There is ethical approval for this study.

**References**

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