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have occurred, mainly in non-vaccinated elephants. This short communication describes an unusual and unexpected fatal case of pox disease in the stillborn calf of a vaccinated female Asian elephant (*Elephas maximus*).

The elephant group of Tierpark Berlin-Friedrichsfelde consisted of five Asian elephants (one male and four females) and seven African elephants (*Loxodonta africana*) (one male and six females). A 15-year-old Asian elephant cow had been vaccinated with the modified vaccinia virus Ankara (MVA) strain and Elstree strain at the age of seven years, but had only developed a very low neutralising antibody titre. The elephant was mated in 1996 and revaccinated with MVA on days 293 and 322 of its pregnancy but gave birth to a well-developed stillborn, male calf with generalised pox lesions. No clinical signs of pox disease were observed in the mother or other elephants of the group.

The stillborn calf had a bodyweight of 117 kg and seemed to have developed normally. The skin showed numerous papules 1 to 1.5 cm in diameter which were slightly raised and light greyish with concentric erythematous rings. Most of these papules were fresh with no tendency to become confluent. Numerous lesions were observed, especially on the trunk, around the eyes, on the ears and on the medial areas of the limbs. Multiple vesicles and ulcers of the mucosa were found in the nasal cavities (Fig 1) and the larynx. The surface of the lungs was covered with a layer of fibrin up to 3 cm in thickness. The lungs showed numerous small, necrotic foci. Discrete erosions and ulcers were observed in the mucosa of the oral cavity, the tongue, the oesophagus, and the entire digestive tract, but especially the stomach (Fig 2) and the large intestine. Some of the larger lesions were covered with thick brown-grey crusts which were raised 5 to 10 mm above the surface of the mucosa. Small, necrotic foci were present in the liver. The capsule of the spleen was swollen, and large erosions were visible. Extensive haemorrhaging was found, particularly in the subepicardial region of the heart. The placenta was not available for examination.

On histological examination of haematoxylin and eosin-stained tissue sections, the lesions in the skin showed acanthosis, ballooning degeneration and acantholysis of the keratinocytes. Serous fluid had accumulated in tiny cystic spaces or vesicles. Typical microscopical changes, for example, eosinophilic homogeneous intracytoplasmic A-type inclusion (ATI) bodies, occurred in the keratinocytes. The lesions in the mucous membranes showed similar histological features. Moreover, severe fibrinous and necrotic bronchiolitis and alveolitis were diagnosed in samples from the lungs. Marked thickening of alveolar walls, accumulation of macrophages, and endothelial necrosis which resulted in haemorrhages were also found. Large, necrotic foci and numerous miliary necrotic foci without peripheral reactions were found in the liver. The spleen and lymph nodes showed a severe lymphocyte depletion.

For virus isolation, homogenates of pox lesions from the skin, spleen and lungs were inoculated on the African green monkey kidney cell lines MA 104 and Vero E6. A cytopathogenic effect (CPE) consisting of large plaques developed 24 to 48 hours after inoculation of the cells. The isolate was named 'Berlin 2'. A subsequent PCR targeting the so-called ATI protein gene was performed (Meyer and others 1997) with DNA extracted from tissue homogenates of the neonate and with DNA of Berlin 2. Subsequent Bgl II digestion of the amplicons revealed a DNA restriction enzyme pattern typical of cowpox viruses. In parallel, the original MVA was treated in the same way. No CPE or plaque formation was observed and the subsequent PCR revealed no amplicon. This is typical of MVA, which has a deletion in the ATI gene and, therefore, cannot be amplified by ATI-PCR. Both the Berlin 2 isolate and MVA were further analysed by a PCR targeting the 14 kD fusion protein (H. Meyer, unpublished data), and subsequent DNA sequenc-

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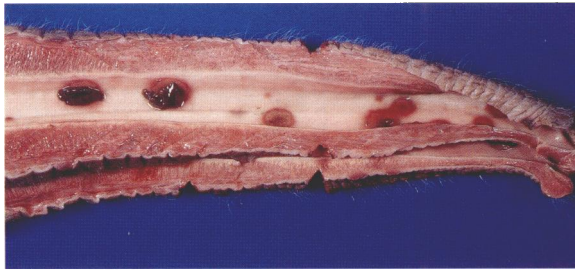
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Cowpox virus infection causing stillbirth in an Asian elephant (*Elephas maximus*)

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POX disease was first described in elephants by Potel and others (1963) after an outbreak in 1960. Since then, more than 26 outbreaks have occurred in Europe (Gehring and others 1972, Pilaski and others 1983, 1988, 1992, Pilaski and Jacoby 1993). Since 1984, most elephants in German zoos have been vaccinated with vaccinia virus (Pilaski and Zhou 1991, Pilaski and others 1995, 1996). However, further outbreaks of pox disease

FIG 1: Multiple vesicles and ulcerations of the mucosa in both nasal cavities in the trunk of the stillborn calf



ing analysis confirmed their identity as cowpox virus and MVA vaccinia virus, respectively (Table 1). A phylogenetic analysis based on ATI sequences (Pilaski and others 1999) revealed that the Berlin 2 virus was closely related to the 'Hannover' strain, isolated in 1980 from an Asian elephant at Hameln near Hannover (Table 2).

On examination by electron microscope, numerous large, homogeneous ATI bodies were observed in the infected Vero cells. The ATI bodies with many occluded viruses formed a dense proteinaceous matrix. Each dense, granular ATI was filled mainly with mature virus. In the virus-replicating cells, irregularly formed 'virus factories' or B-type inclusion (BTI) bodies were consistently observed. The BTI bodies showed all stages of viral morphogenesis and many immature viral particles. The ultrastructural features of adsorption, penetration, uncoating and assembly were consistent with that of the cowpox viruses. In the infected tissue culture cells a pronounced virus production was found. In addition to ATI bodies, numerous extracellular mature viruses were observed.

Serum from all elephants were tested for antibodies against orthopoxvirus in a plaque reduction test using MA 104 cells and 100 TCID₅₀ vaccinia virus (Czerny and others 1997). All sera had neutralising antibodies with titres ranging from 1:8 to 1:512. Neutralising antibody titres of 1:8 were detected in 10 serum samples from the female elephant in the present

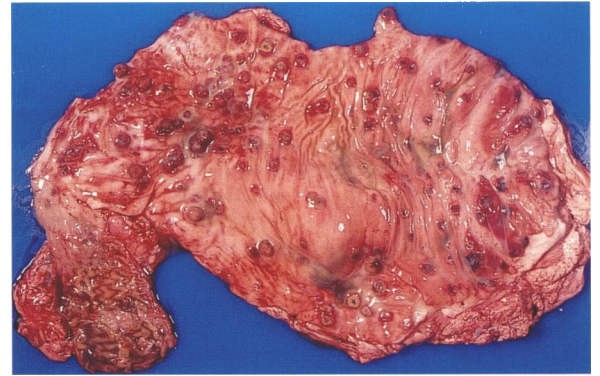


FIG 2: Severe circumscribed, erosive ulcers in the mucosa of the stomach of the stillborn calf

case, collected between June 1, 1994, and November 26, 1997. A significant seroconversion was found in the elephant cow with a titre from 1:256 in December 1997, and with 1:1024 in January 1998.

This is the first report of a congenital cowpox virus infection in elephants. Several cases of fatal generalised vaccinia virus infection in human fetuses were reported during the smallpox eradication programme (Green and others 1966, Vorst and Gaillard 1983). Congenital fatal swinepox virus infections in pigs were observed in Nigeria (Olufemi and others 1981), in the Netherlands (Borst and others 1990) and in England (Paton and others 1990). Moreover, it has been shown that congenital ectromelia infection caused by the ectromelia virus was endemic in farms of silver foxes (*Vulpes vulpes*) and mink (*Mustela lutreola*) in the Czech Republic (Mahnel and others 1993). This fatal infection was congenitally transmitted and resulted in stillbirths or birth of sick neonates. Adult animals were usually free of clinical signs.

In the present report, the diagnosis of a generalised congenital cowpox virus infection of an elephant fetus was established by the presence of typical lesions containing cytoplasmatic ATI bodies, the isolation of an orthopoxvirus from the lesions and the identification of the causative agent as cowpox virus by PCR. Since the calf was fully developed and showed no signs of maceration, it was concluded that it had become infected three to four weeks antepartum and that the infection was not a recrudescence of a latent infection of the maternal placenta. The mode of infection in this case showed marked differences compared with that described in adult elephants. The occurrence of advanced pox lesions in the respiratory and digestive tracts and of early pox lesions

TABLE 1: PCR results following amplification of viral DNA derived from lesions of the skin of a stillborn calf and from the vaccine strain used in vaccinating the elephant cow

	Tissue	Elephant Isolate	MVA -strain vaccine
ATI gene PCR	Positive	Positive	Negative
Bg1 II pattern	Cowpox-like	Cowpox-like	ND
14kD fusion protein PCR	Positive	Positive	Positive
DNA sequence	Cowpox-like	Cowpox-like	MVA

ATI A-type inclusion, MVA modified vaccinia virus Ankara, ND No data

TABLE 2: Cowpox virus strains isolated from zoo-kept mammals in Germany (A) and reference orthopoxvirus species (B) used for comparative genome analysis

Virus strain	Orthopoxvirus	GenBank accession number	Species	Year of outbreak	Reference
A EP-Augsburg	Cowpox virus		<i>Elephas maximus</i>	1971	Gehring and others (1972)
EP-Hannover	Cowpox virus		<i>E maximus</i>	1980	Pilaski and others (1983)
EP-Berlin 1	Cowpox virus		<i>E maximus</i>	1986	Pilaski and others (1988)
EP-Erfurt	Cowpox virus		<i>Loxodonta africana</i>	1988	Pilaski and others (1992)
EP-Lehrte	Cowpox virus		<i>E maximus</i>	1994	Pilaski and others (1995)
EP-Cologne	Cowpox virus		<i>E maximus</i>	1994	Pilaski and others (1996)
LP-Leipzig	Cowpox virus		<i>Lama glama pacos</i>	1994	Schüppel and others (1997)
EP-Berlin 2	Cowpox virus		<i>E maximus</i>	1998	Wisser and others (1998)
B Brighton	Cowpox virus	D00319	<i>Homo sapiens</i>	1937	Funahashi and others (1988)
M-1	Mousepox virus	X69325	<i>Mus domesticus</i>		Osterrieder and others (1994)
Bangladesh-75	Variola virus	L22579	<i>H sapiens</i>	1975	Massung and others (1993)
Western reserve	Vaccinia virus	X57318			Amegadzie and others (1991)
Copenhagen	Monkeypox virus	V84503	<i>Macaca irus</i>	1957	Neubauer and others (1998)

in the skin suggests that the infection was initially established in the intestine and later spread to the epidermis.

One explanation for the fatal infection of the fetus could be the very low neutralising antibody titre of the mother. A titre of 1:8 may have been sufficient to protect the cow from developing symptoms during an infection but may not have protected the fetus. The mother may have become viraemic after an orthopoxvirus infection and, during the viraemia, the fetal membranes may have become infected, as occurs in cases of congenital vaccinia virus infections (Green and others 1966).

Maternal antibodies such as immunoglobulin G or immunoglobulin M cross the placenta rather poorly so that the fetus is not fully protected by maternal antibodies while in utero. The human placenta gives some protection against maternal infections, including most bacteria, but it does not prevent the passage of a number of viruses, some bacteria and protozoa (Wigglesworth 1992). These observations indicate that in a case of an orthopoxvirus infection, the mother remains healthy while the fetus faces the infection unprotected. The fetus is extremely sensitive to several types of viral infection, especially during the last months of pregnancy.

It is currently known that wild-living rodents are the primary carriers of cowpox virus in Europe (Pilaski and Jacoby 1993, Tryland and others 1998). Zoo-kept elephants can come into contact with virus easily, since hay and straw can be contaminated with the infected urine and faeces of rodents. Twelve of 25 wild-living red foxes (*Vulpes vulpes*) shot in the Tierpark Berlin-Friedrichsfelde area in 1997 and 1998, were seropositive for orthopoxvirus with titres ranging from 1:2 (five foxes) to 1:32 (three foxes). A titre of 1:4 was also detected in one stone marten (*Martes martes*). Compared with other regions, the prevalence in red foxes of nearly 50 per cent is high. Müller and others (1996) reported a prevalence rate of 16 per cent positive and 15 per cent suspected positive fox sera from Brandenburg state which is adjacent to Berlin. These results suggest that red foxes are good indicators of the level of cowpox virus infection in wild-living rodent populations, as rodents are part of the diet of foxes.

The breeding of elephants in zoological gardens has become increasingly important as the regulations issued by the Convention on International Trade in Endangered Species of Wild Fauna and Flora bans the import of wild-caught elephants. This fatal case of cowpox infection is, therefore, of concern to the Tierpark Berlin as it affected the first birth of an elephant in captivity in this zoo for 60 years.

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