An outbreak of encephalomyocarditis-virus infection in free-ranging African elephants in the Kruger National Park

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ABSTRACT

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A cluster of four deaths in late December 1993, marked the onset of an outbreak of disease of African elephants (*Loxodonta africana*) in the Kruger National Park (KNP) in South Africa, which has an estimated population of 7 500 elephants. Mortalities peaked in January 1994, with 32 deaths, and then declined steadily to reach pre-outbreak levels by September, but sporadic losses continued until November. During the outbreak altogether 64 elephants died, of which 53 (83%) were adult bulls. Archival records revealed that, in addition to the usual losses from known causes such as poaching and intraspecific fighting, sporadic deaths from unexplained causes had, in fact, occurred in widely scattered locations from at least 1987 onwards, and from that time until the perceived outbreak of disease there had been 48 such deaths involving 33 (69%) adult bulls. Carcases had frequently become decomposed or had been scavenged by the time they were found, but seven of eight elephants examined early in 1994 had lesions of cardiac failure suggestive of encephalomyocarditis (EMC)-virus infection, and the virus was isolated from the heart muscles of three fresh carcases. The results of tests for neutralizing antibody on 362 elephant sera collected for unrelated purposes from 1984 onwards and kept frozen, indicated that the virus had been present in the KNP since at least 1987. Antibody prevalences of 62 of 116 (53 %), 18 of 139 (13%) and seven of 33 (21%) were found in elephants in three different regions of the KNP in 1993 and 1994. Studies had been conducted on myomorph rodents in the KNP for unrelated purposes since 1984, and trapping attempts were increased during the perceived outbreak of disease in elephants. There was a striking temporal correlation between the occurrence of a population explosion (as evidenced by markedly increased catch rates per trap-night) and a surge in prevalence of antibody to EMC virus in rodents, and the occurrence of the outbreak of disease in elephants.

Keywords: Encephalomyocarditis virus, African elephant, *Loxodonta africana,* rodents, Kruger National Park, South Africa

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INTRODUCTION

Encephalomyocarditis virus is a member of the genus *Cardiovirus* of the family *Picornaviridae*, with a worldwide distribution. Various names, including Columbia-SK, MM, ME (maus Elberfeld) and Mengo, were given to early isolates, but it was found that they all belonged to the same serotype as an agent isolated from a chimpanzee, which caused encephalitis and myocarditis in laboratory rodents; hence the term encephalomyocarditis (EMC) was adopted for the virus (Murnane 1981; Zimmerman 1994). The first two

isolations, involving the Columbia-SK and MM strains, were made from material derived from poliomyelitis patients in the USA in 1940 and 1943, and consequently it was suspected that the virus was associated with this fatal nervous disease of humans. In retrospect, however, it was concluded that EMC virus had probably been a contaminant of the laboratory rodents used in the poliomyelitis investigations. Although there have been a few reports which link the virus to transient meningitis and encephalomyelitis, the cumulative evidence indicates that the vast majority of infections in humans are asymptomatic or unrecognized (Tesh 1978; Murnane 1981; Zimmerman 1994).

The presence of EMC virus, or antibody to it, has been demonstrated in surveys on wild myomorph rodents (rats and mice) in many countries and on all continents. The virus has an extremely wide host range and, although rodents have often been suggested to be the source of infection in outbreaks of disease in other species, it has also been argued that they are merely indicators of the general circulation of the virus in a wide range of animals in a given environment (Tesh & Wallace 1978; Zimmerman 1994). The occurrence of disease, including sudden death associated with myocarditis, abortion, stillbirth and foetal death, and mummification, has been reported most frequently in pigs. Descriptions of disease in other domestic animals are rare despite the detection of high prevalences of antibody on occasion (Tesh & Wallace 1978; Murnane 1981; Zimmerman 1994). Outbreaks of disease have been reported in captive wild animals in zoos and breeding colonies, particularly in primates which develop acutely fatal myocarditis or, less commonly, encephalitis with paralysis (Wells, Gutter, Soike & Baskin 1989; Hubbard, Soike, Butler, Carey, Davis, Butcher & Gauntt 1992; Zimmerman 1994). The virus was incriminated of causing the deaths from myocarditis of eight captive African elephants (*Loxodonta africana*) in two zoos in the USA, and another in a zoo in Australia, and was suspected of causing the deaths of lions fed meat from the elephant carcases in one instance (Simpson, Lewis & Gaskin 1977; Seaman & Finnie 1987). The present communication, which describes an outbreak of disease which was observed in the Kruger National Park (KNP) in South Africa in 1993–1994, constitutes the first report of fatal infection with EMC virus recorded in free-ranging African elephants.

HISTORY OF THE OUTBREAK

The KNP is situated in the eastern Transvaal, along the north-eastern border of South Africa with Mozambique, between the latitudes of 22°20′S and 25°32′S. It is 1 948 528 ha in extent and has a north-south length of about 350 km and an average east-west width of 65 km. It is divided into four administrative regions: the Far North, the North, the Central and the South regions, each of which is subdivided into sections controlled by rangers (Fig. 1). The KNP supports an elephant population which is maintained at about 7500 individuals through the conducting of an annual aerial census and selective culling or the translocation of excess herds or family groups.

The occurrence of overt disease or mortalities in animals in the KNP is monitored routinely by sectional rangers and their game guards, and veterinary and other officials. Attention is often drawn to the carcases of large animals by the observation of vulture activity, but elephant mortalities are encountered infrequently, usually no more than once or twice a month, except when there is an occasional upsurge of poaching in a particular area, as occurred in 1992 (Fig. 2). At the end of December 1993, a cluster of four elephant deaths was noticed in the Far North and North regions of the KNP, and by January 1994, losses were also recorded in the Tshokwane and Lower Sabie areas in the south of the Central region. Most deaths were recorded in January, after which losses declined steadily to reach pre-outbreak levels by September. Altogether 64 elephants are known to have died from otherwise unexplained causes during the period of the outbreak (Fig. 2). The animals that died were overwhelmingly adult bulls, but included at least nine cows and two calves. The deaths occurred in widely scattered locations, but were clustered mainly in the Shangoni-Shingwedzi-Woodlands area in the North region, and in the Tshokwane-Lower Sabie area in the south of the Central region (Fig. 1).

Carcases had often become decomposed, or had been scavenged by lions and hyenas by the time that they were located, rendering it difficult to perform proper autopsies or laboratory investigations. Nevertheless, poaching and anthrax were eliminated as possible causes of the deaths at an early stage of the investigation, and bacteriological and toxicological studies proved futile. Suspicion turned to EMC virus, particularly since myocarditis was found to be the most striking lesion at autopsy, and specimens taken from three fresh carcases, including that of an elephant shot for examination, were submitted for virological investigation. Serum samples which had been collected from elephants in the KNP over many years for unrelated purposes and kept in frozen storage, were made available for testing for antibody to EMC virus, and further serum samples were collected from a few lions and from humans involved in the investigations.

At the end of November 1993, shortly before the elephant mortalities were recognized, sick tree squirrels (*Paraxerus cepapi*) were seen near the Luvuvhu River in the Far North region of the KNP. Six sick individuals and a decomposed carcase were found lying on roads over a 3 d period and within a radius of 10 km. The sick squirrels were unwilling to move,

appeared to be sluggish and ataxic when approached, and four of them were caught by hand for examination. At the same time two decomposed squirrel carcases were found on a main road in the vicinity of Tshokwane, near the southern end of the Central region. It could be deduced from the numbers of rats and mice encountered on the roads, particularly at night, that there had been a population explosion of myomorph rodents in the Far North region. Flattened and decomposed myomorph rodent carcases were also seen on the roads in daylight, but it was impossible to tell whether these had died from illness or had merely been killed by motor vehicles. Questioning of officials revealed that a widespread increase in the rodent population in the KNP had indeed been noticed at least a month earlier, and that the subjective impression had been gained that raptors, owls and snakes, which prey on rodents, had also been more evident than usual. Anecdotal evidence was obtained from a malaria expert who had visited Mozambique in his official capacity in November 1993, that there had been a widespread population explosion of rodents in that country as well, and that rodenticide was being distributed to members of the public (F.C. Hansford, personal communication 1994). During mid-1994 there were news-media reports that a plague of rodents was destroying crops in the Limpopo Valley to the west of the KNP. Fortuitously, serum and organ samples had been collected from myomorph rodents in the KNP at intervals from December 1984 to October 1993, for an unrelated study of arena- and hantaviruses, and residual serum samples which had been kept in frozen storage remained available for EMC tests. Further rodent specimens were collected specifically for EMC investigation in the KNP from November 1993 to June 1994, starting with the area where the sick squirrels had been found. For comparative purposes, serum samples were also obtained from other locations in the country where health inspectors routinely sample rodent populations in order to monitor for possible bubonic-plague activity.

MATERIALS AND METHODS

Reference viruses and antisera

The South African reference strain of EMC virus, AN7402, was isolated from a multimammate mouse, *Mastomys natalensis* sensu lato (probably *M. coucha*) (Gordon & Watson 1986), caught in the grounds of Rietfontein Hospital, Edenvale, in a survey in 1961 [National Institute for Virlogy (NIV), unpublished records 1953–1994], and hyperimmune-mouse ascitic fluid (HMAF) was prepared as described elsewhere (Sartorelli, Fischer & Downs 1966). The isolate was identified in cross-neutralization tests with the Mengo strain and an unspecified Hawaiian strain of EMC virus apparently obtained from the Yale Arbovirus Research Unit, New Haven, Connecticut, USA (NIV, unpublished records 1953–1994). The arenavirus, Mopeia, was isolated at NIV in 1972 from *Mastomys natalensis* sensu stricto rodents collected in Mozambique (Wulff, McIntosh, Hamner & Johnson 1977); Hantaan virus, strain 76118, was obtained in 1984 from the US Army Medical Research Institute for Infectious Diseases, Fort Detrick, Frederick, Md, USA, and a reference strain of reovirus 3 was isolated at NIV (NIV, unpublished records 1953–1994). Reference antibodies (HMAFs) to these latter viruses were prepared as for EMC virus.

Mortalities and specimens

Figures for elephant mortalities observed in the KNP since the second quarter of 1987 were obtained from archival records of sectional rangers' diary reports, and by submission of a specific questionnaire to rangers for the period November 1993 to April 1994.

Autopsies were performed on eight elephants, including a sick animal killed for examination. Macroscopic changes were recorded and in five instances tissue samples of the brain, heart, lungs, liver, kidney, spleen, lymph nodes, skeletal muscles, stomach and small and large intestines, were fixed in 10% buffered formalin for histopathological examination. Unfixed tissue samples were taken for bacteriological and toxicological examination and, in three instances, for virology as well. The specimens for virological investigation came from the carcase of an adult cow found at Mlondozi in the south of the Central region of the KNP in January 1994, from a bull found a short distance away at Rietpan in February and from a sick bull shot for examination in the Vlakteplaas area of the North region in January. The sick bull had separated itself from the herd, was disinclined to move from the shade of a large tree, and manifested respiratory distress, and irascibility when approached.

Serum samples which had been collected from elephants in the KNP from 1984 to mid-1994 in unrelated studies or in capture and culling operations, and stored initially at –40 °C and later at –70 °C, were tested for antibody to EMC virus as described below. Serum samples were also collected in 1994 from a lioness and two sub-adult cubs which were immobilized and bled when found feeding on an elephant carcase in which lesions compatible with EMC-virus infection were seen, and re-bled 6 weeks and 3 months later. Tracking of the lions was facilitated by the fitting of a radio-collar to the lioness on the first occasion. Serum was also collected from the heart blood of a 6-month-old male cub found dead with bacterial pneumonia (*Klebsiella* spp.), and from humans in the KNP and at NIV, most of whom had been directly involved in the EMC investigations.

Rodent traps (Willan 1979) baited with a mixture of rolled oats and peanut butter were set at dusk and

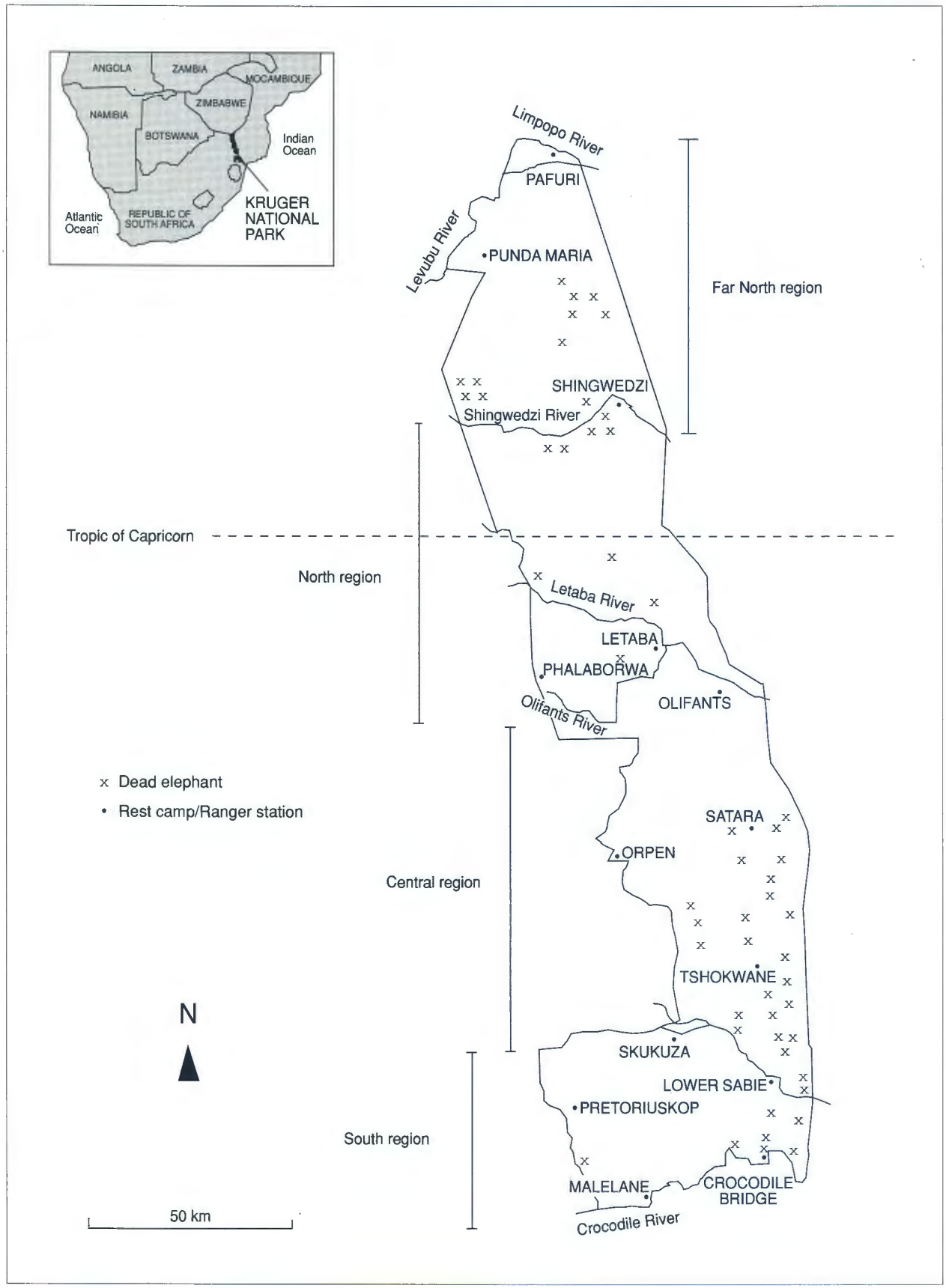

FIG. 1 Locality map of the Kruger National Park and distribution of elephant deaths

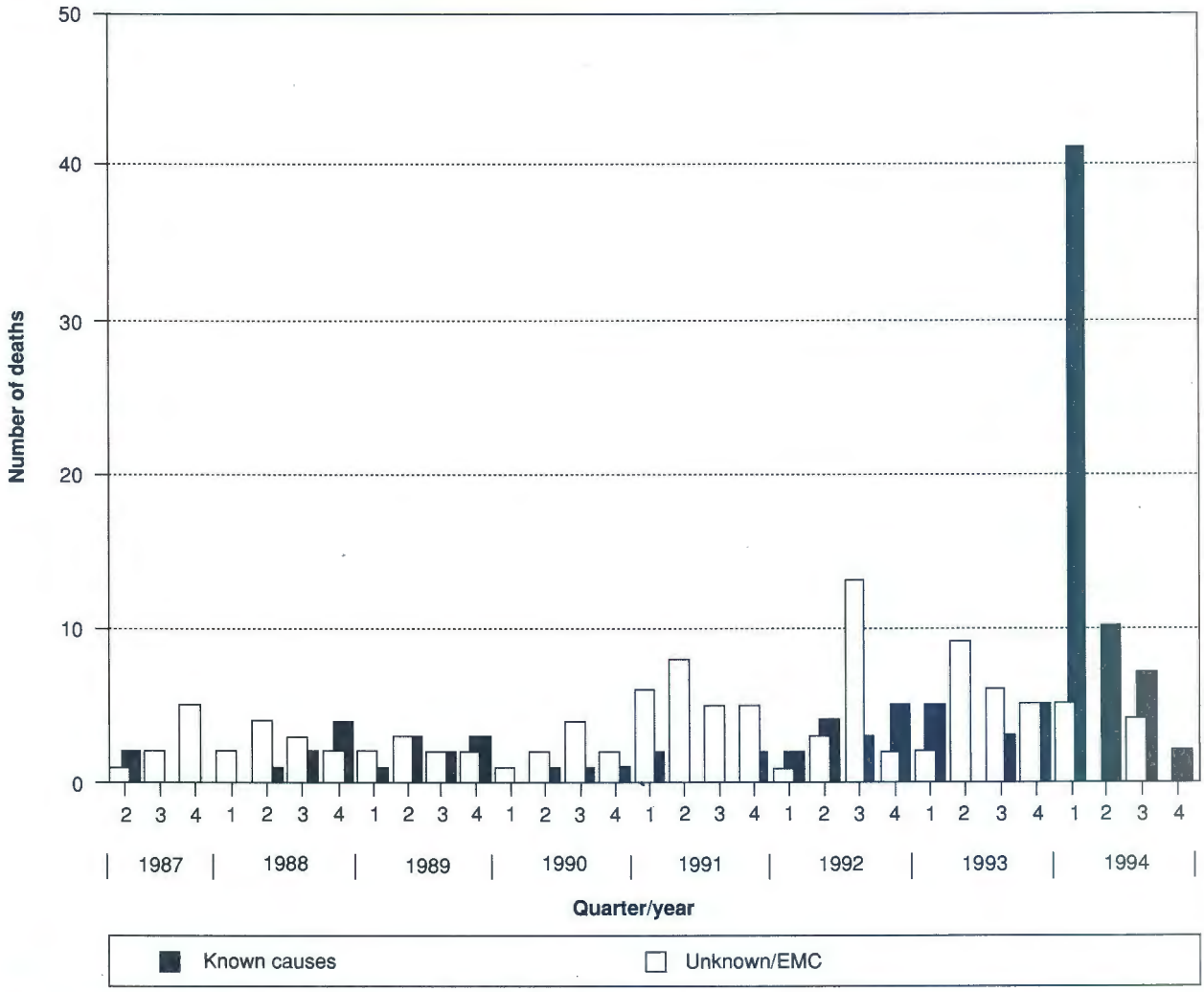

FIG. 2 Recorded elephant deaths in the Kruger National Park 1987–1994

collected at sunrise. Rodents were caught alive, anaesthetized with ether and exsanguinated by cardiac puncture. Then brain, spleen, liver, kidney, heart and lung samples were collected in cryotubes for virological investigation. Serum and organ samples were held in a –20°C freezer or in a liquid-nitrogen flask in the field, and transferred to a –70°C freezer in the laboratory. The sick squirrels found near the Luvuvhu River were euthanased, and serum plus organ samples were collected for virological and histopathological investigation.

Histopathological investigations

Tissue sections stained with haematoxylin and eosin (HE) were examined for lesions, and replicate sections were stained for the demonstration of EMC-virus antigen by the immunocytochemistry technique of Haines & Chelack (1991), with a 1/1000 dilution of HMAF, and final detection was made with an avidin-biotin-complex reaction kit, and 3,4,3,4' tetra-aminobiphenyl hydrochloride and Vector VIP colour reagents (Vector Laboratories Inc., Burlingame, Ca 894010, USA).

Virus isolation and identification

Approximately 10% homogenates of tissue samples were prepared in cell-culture medium and clarified by centrifugation for 15 min at 3000 *g* on a bench centrifuge. The supernatants were inoculated intracerebrally in day-old mice, and into BHK21, CER and Vero-cell cultures for the isolation of the virus. Tissue samples from elephants were tested individually, but the various organ samples of each rodent were tested as a pool. Isolates of EMC virus from elephants were identified by neutralization tests in cell cultures with reference HMAF as described below, while reovirus-3 and Mopeia-virus isolates from rodents were identified by immunofluorescence tests performed on the brains of inoculated mice which succumbed, and on infected cell cultures.

Since problems were encountered with reovirus and Mopeia-virus infections during attempts to isolate

EMC virus from rodent tissue, recourse was made to reverse transcription and the polymerase chain reaction (RT-PCR) to demonstrate the presence of EMC-virus nucleic acid in arbitrarily selected specimens from antibody-positive and -negative individuals, employing previously described methods and primer sequences (Kyu, Matsumori, Sato, Okada, Chapman & Tracy 1992). The RT-PCR procedures were also applied to squirrel and elephant tissue, and EMC-virus-infected and non-infected cell cultures were used as controls.

Antibody tests

An isolate of EMC virus obtained from elephant tissue in the present study, and designated SPU19/94, was passaged a few times in Vero-cell cultures to prepare a stock of cytopathic virus for use in serological tests, and this was stored in small volumes at –70 °C.

Antigen spot slides for indirect immunofluorescence (IF) tests on rodent sera were prepared on 8-well teflon-coated slides (Sterilab Services CC, Johannesburg, South Africa) from a mixed suspension of Vero cells infected with EMC isolate SPU19/94 and non-infected cells, as described previously for Crimean-Congo haemorrhagic-fever virus (Shepherd, Swanepoel, Shepherd, McGillivray & Searle 1987). Antigen slides were prepared in the same way with Hantaan and Mopeia viruses to test rodent sera for group-reactive antibodies to hantaviruses and African arenaviruses. Sera were screened at a dilution of 1/8 in IF tests performed with anti-mouse immunoglobulin fluorescein conjugate (Zymed Laboratories Inc., San Francisco, USA), and positive specimens were titrated to end-point in doubling dilutions.

Neutralization tests for antibody to EMC virus were performed on elephant, lion and human sera, and on 66 selected rodent sera for comparison of the results with those obtained in IF tests. Rodent sera to be tested for neutralizing antibody were arbitrarily selected to include specimens which lacked antibody to EMC virus in IF tests, as well as specimens with low and high IF titres. Test sera were inactivated at 59 °C for 30 min and serial twofold dilutions from one of eight to one of 1024 were prepared in duplicate in 0,1 mℓ volumes of medium (Leibovitz 1963) containing 5% foetal calf serum, in flat-bottomed 96-well cell-culture microplates. Equal volumes of virus suspension containing a calculated 100 doses of cell culture that are 50% infective ($TCID_{50}$) were added to each well and the serum-virus mixtures incubated for 1 h at room temperature (22 °C) before they were seeded with 2 x 10^4 cells per well in 0,025 mℓ of medium. The plates, with loose lids, were sealed in humid chambers and the tests read after 7 d of incubation at 37 °C. Antibody titres were recorded as the reciprocals of the highest serum dilutions at which neutralization occurred in both replicates. Control virus titrations were performed in six replicates per tenfold dilution and titres estimated by the method of Kärber (1931). In neutralization tests for the identification of isolates, serial tenfold dilutions of virus were incubated in duplicate with equal volumes of a one-of-ten dilution of EMC HMAF or non-immune ascitic fluid, inoculated into six replicate cell-culture wells per dilution of virus, and $\log_{10}$ neutralizing indices (NIs) recorded as the differences in titre between the titrations with immune and non-immune ascitic fluids.

RESULTS

Mortalities and gross pathology

The numbers of elephant deaths recorded in the KNP during each quarter from April 1987 to December 1994, excluding animals killed in culling and capture operations, are presented graphically in Fig. 2. A total of 111 elephants [99 (89%) adult bulls, eight cows and four calves] died from causes which could readily be determined by rangers in the field. These included, for instance, elephants killed by poachers, and animals which had to be destroyed after they had either been wounded by poachers, or injured by snares or intraspecific fighting. A further 112 elephants [86 (77%) bulls,16 cows and ten calves] died from causes which could not be established in the field, and these included individuals in which a diagnosis of EMC infection had been confirmed in 1994. The predominance of adult bulls among the elephants that died of unexplained causes persisted throughout the recorded period: bulls constituted 33 of 48 (69%) of the animals that died before the perceived outbreak of disease occurred at the end of December 1993, and 53 of 64 (83%) of those that died afterwards. Unexplained mortalities were recorded from at least 1987 onwards (Fig. 2), but many were seen as sporadic deaths in widely scattered locations, and hence systematic investigation was not undertaken until the clustering of four deaths at the end of December 1993, implied the occurrence of an outbreak of disease associated with a specific etiology. A peak number of 32 unexplained deaths occurred in January 1994, after which the losses declined to reach pre-outbreak levels by September, but continued to occur until November (Fig. 2).

Cardiac failure appeared to be the most likely cause of death in all of the elephants autopsied in 1994, except in the instance of the animal killed for examination, in which no overt lesions were found. Hydrothorax, hydropericardium and severe ascites (up to 50 ℓ of straw-coloured fluid in one instance) were observed in the remaining seven elephants examined. Heart lesions included disseminated petechial or ecchymotic haemorrhages on the epicardium, with a streaky appearance. The myocardium had a mottled appearance, with multifocally distributed pale, streaky ar-

eas, and disseminated small haemorrhages in some instances. Pale streaks or foci were observed in the endocardium as well.

Lungs were congested and oedematous, with interstitial oedema being particularly prominent, and cut surfaces exuded pale, frothy or fibrinous fluid. Livers appeared slightly swollen and congested in most instances, but in two animals they had a mottled appearance. Pronounced congestion was evident on the visceral and parietal surfaces of the peritoneum in four animals, with accompanying oedema of the mucosa and subserosa of the stomach and the intestines. Mediastinal and mesenteric lymph nodes were soft and swollen, and exuded watery fluid on cut surfaces. The brain appeared to be oedematous and mildly congested in all cases examined.

Sick squirrels were thin and lacked body fat, but no other gross abnormalities were noticed. Trapped myomorph rodents generally lacked overt abnormalities, apart from lesions associated with extra-intestinal stages of helminth parasites. However, no specific inspection was made for heart lesions and, in any event, these would probably have been masked by the lesions produced by cardiac puncture during exsanguination.

Histopathology

Histopathological lesions compatible with EMC-virus infection were observed in the tissue of four of five elephants examined, the exception being the animal killed for examination. The most consistent and marked lesions occurred within the myocardium. Disseminated focal lymphocytic myocarditis, accompanied by necrosis of myocytes, occurred throughout the heart with no specific anatomical predilection, although the lesions varied in severity in different elephants. Mild lesions involved single or small groups of myocytes and, in most instances, there was a scant mononuclear infiltration around affected myocytes, occasionally extending into necrotic fibres, but marked inflammatory infiltration was evident in locally extensive necrotic areas in two elephants. In addition, there were foci or locally extensive areas of degeneration characterized by disorganization and disintegration of myofibrils, the presence of contraction bands and atrophy or hypertrophy of myocytes with nuclear enlargement. Varying amounts of lipofuscin occurred in a juxtanuclear position in the myocytes of some animals. Scattered haemorrhages and pronounced patches of congestion occurred in the myocardium, and epi- and endocardium.

Alveolar and interstitial oedema and severe congestion were consistently present in the lungs, while the liver of one animal manifested acute hypoxic centrilobular coagulative necrosis and sinusoidal dilation. Severe oedema of the meninges and the neuropil was evident in brain sections. The skeletal muscle of one animal manifested mild degeneration and regeneration of myofibres. There was severe lymphoid atrophy of lymphoid tissues. Marked oedema of the serosa and submucosa occurred in the colon and stomach.

Immunostaining for EMC-virus antigen was performed only on specimens originating from three fresh elephant carcases. Antigen was demonstrated in two out of three instances, the exception being the elephant which was killed for examination. Antigen was found only in and surrounding necrotic foci in the myocardium, and most of the reactivity in myofibrils occurred at the periphery of such foci. The immunoreactivity was finely granular, never abundant and restricted to the myocytes.

No abnormalities were observed in sections of brain, spleen, liver, kidney and lung tissue from sick squirrels, but it had been omitted to fix heart tissue for histopathological investigation.

Virology

Virus was isolated in mice and cell cultures from heart tissue from all three elephants from which specimens had been submitted for virological examination, including the elephant killed for autopsy, and the isolates were identified as EMC virus in neutralization tests with the reference HMAF ($\log_{10}$ NIs $\geq 5,0$).

Rodent specimens from the KNP, collected and tested from 1984 to 1992, yielded isolations of the southern African arenavirus, Mopeia, in mice and/or cell cultures, but no other viruses (NIV, unpublished records 1953–1994). The squirrel- and other rodent-tissue specimens which were collected in the Far North of the KNP at the end of November 1993, were subjected to high ambient temperatures for a few days when a freezer malfunctioned in the field, and no isolations of virus were made from these specimens. Suspensions of other specimens collected from rodents in 1993 and 1994 for the EMC project killed inoculated suckling mice in a high proportion of instances and, by performing IF tests on harvested mouse brains and cell cultures, it was established that much of the tissue yielded reovirus 3, while there were also four isolations of Mopeia virus. Hence, attempts to isolate virus from rodent tissue were abandoned, and RT-PCR tests for the presence of EMC-virus nucleic acid were performed on a few selected specimens instead. A PCR product of expected size (574 bp) was demonstrated in ethidium-bromide-stained agarose electrophoresis gels, in six of ten specimens from *M. natalensis* rodents which had IF antibody to EMC virus, and which came from four widely separated locations in the KNP, and in none of eight specimens from individuals which came from the same locations but lacked antibody. The RT-PCR test was also positive on two of three elephant-heart specimens submitted for virological examination, with the

exception relating to the animal killed for examination. Inconclusive RT-PCR results were obtained on autolysed tissue of sick squirrels: findings were initially positive but could not be reproduced. The presence of EMC-virus nucleic acid was demonstrable by RT-PCR in infected control-cell cultures, but not in uninfected controls.

Serology

The results of IF tests for antibody to EMC virus on 416 rodent sera from the KNP are summarized in Table 1. The earliest seropositives detected by IF occurred in three of 13 rodents (one *Rattus rattus* and two *Aethomys chrysophilus*) caught at Skukuza in the South region in September and October 1993, shortly before the outbreak of mortalities in elephants came to the attention. No seropositives were recorded in 23 rodent sera collected in the Far North at the end of November 1993, including samples from two sick squirrels, but from January 1994 onwards, IF antibody to EMC virus was found to be widely prevalent in rodent sera in the KNP, including the location where the squirrels had been caught. Rodents were trapped at a total of 30 locations in the KNP in 1994, and at only five sites close to Shingwedzi camp in the North antibody was not detected. The highest prevalence of antibody occurred in *M. natalensis*, which was also the most numerous rodent, but seropositives were recorded in other species (Table 1). Peak prevalence of antibody to EMC virus occurred in March 1994, and then declined slightly. As on past occasions, antibody to Mopeia virus was found to be widely prevalent in KNP rodents (to be reported elsewhere), but no antibody to Hantaan-virus antigen was detected.

Rodent trapping rates (rodents caught per trap-night) recorded in the KNP since 1984 are summarized in Table 1. Catch rates were not recorded on all occasions, nor were all rodents caught, necessarily harvested. For instance, only 26 of 75 rodents caught at one particular site in 1994, were harvested. Rodents were trapped at multiple sites throughout the KNP and in various seasons in all of the reported years except 1993, and on no occasion prior to 1993 was a catch rate in excess of 20% recorded at any site. In contrast, the catch rate exceeded 50% in the North region in November 1993, and was greater than 100 % at a few sites in 1994: some rodents were caught while traps were still being set, and up to three individuals were found together in traps. The rodent populations in the KNP appeared to decline rapidly after June 1994, and trapping attempts in July and August at two sites where there had previously been high catch rates, proved futile (data not included in the catch rates shown in Table 1).

Rodent catch rates in excess of 50% were recorded in the course of bubonic-plague-monitoring activities in many districts throughout South Africa during the first half of 1994, and IF antibody to EMC virus was found in 34 of 495 sera, and in 12 of 31 districts tested outside the KNP (KwaZulu-Natal districts were not sampled). The highest prevalences of antibody were recorded in the Bloemhof and Delareyville districts, and low prevalences were found in the Johannesburg, Frankfort, Kroonstad, Boshoff, Dewetsdorp, Tarkastad, Cradock, East London, Bathurst and Alexandria districts. Species in which antibody was found outside the KNP included *M. coucha*, *Rhabdomys pumilio*, *Otomys irroratus* and *Malacothrix typica*.

Neutralization tests on rodent sera were slightly more sensitive than IF tests for demonstrating antibody to EMC virus: 48 of 66 sera lacked antibody by both methods (titres < 8), five sera which were negative in the IF test had low titres (8–32) in neutralization tests, while the remaining 13 sera had either intermediate (64–256) or high (512–1 024) titres by both methods. It is notable that one rodent collected in 1987, an *A. chrysophilus* caught in the Far North of the KNP, had a neutralizing titre of eight to EMC virus, whereas antibody was first detected by IF in sera collected in 1993.

The results of neutralization tests on 362 elephant sera collected in the KNP during 1984–1994 are summarized in Table 2. Antibody was detected in sera collected from 1987 onwards, but samples taken before 1993 came from individuals or small numbers of animals sampled within herds, so that it is difficult to draw conclusions on the general prevalence of antibody. One elephant bled in 1989 when it developed respiratory distress with frothing at the nares while being immobilized and captured, had a titre of 128, and the titres in the remaining eight seropositives bled before 1993 had a range of 8–32. Larger numbers of elephants were sampled in culling and capture operations in 1993 and 1994, which were conducted in the North region in May and June 1993, and in the Far North plus the Malelane area in the extreme south of the KNP in May and June 1994. Neutralizing antibody titres recorded during these 2 years, had a range of 8 $\geq$ 1 024, with a geometric mean of $\geq$ 37. Antibody was found in 62 of 116 (53 %) sera and in ten of 11 herds in the North region in 1993, and in 62 of 107 (58%) of individuals within the seropositive herds. The elephants bled in 1993 were classified as either calves $\leq$ 7 years old, juveniles 8–15 years old, or adults, based on height measured at the shoulder. The prevalence of antibody in females, 61% (43 of 71), was somewhat higher than in males, 42% (19 of 45) (relative risk of positivity in females 1,43), and the immune rates in the various age groups ranged from 65% (28 of 43) in adults, through 48% (25 of 52) in juveniles to 43% (nine of 21) in calves, but none of these differences is significant ($P \geq 0,05$). In 1994, antibody was found in 25 of 172 (15%) sera and in 12 of 25 herds tested. Most of the sera collected in 1994 came from the Far

TABLE 1 Results of indirect immunofluorescence tests for antibody to encephalomyocarditis virus in rodent sera and annual trapping rates of rodents in the Kruger National Park

Species	1984	1986	1987	1989	1990	1993	1994
Paraxerus cepapi		0/2[a]		0/1		0/2	
Graphiurus murinus							0/7
Aethomys chrysophilus	0/9	0/9	0/8	0/10	0/15	2/13	1/34
Aethomys namaquensis		0/1	0/2		0/1		
Lemniscomys griselda		0/1	0/1		0/1		
Mus minutoides	0/5						0/3
Mastomys natalensis	0/42	0/4	0/16	0/11		0/10	100/264
Rattus rattus	0/6	0/2	0/3	0/7		1/1	0/4
Saccostomus campestris	0/7	0/3	0/4			0/5	2/41
Steatomys pratensis	0/2						
Tatera leucogaster	0/7		0/3	0/8	0/14	0/4	2/63
Thallomys paedulcus	0/7		0/18	0/2	0/1	0/1	
Total	0/85	0/22	0/55	0/39	0/32	3/36	105/416
Trapping rate	105/931[b]		29/241	61/1684	45/1534	19/35	530/947
Trapping rate %	11		12	4	3	54[c]	56

[a] Sera positive/sera tested
[b] Rodents caught/trap-night; not recorded at all sites; not all rodents sampled—see text
[c] End of November 1993, rodent population explosion first documented

TABLE 2 Results of neutralization tests for antibody to encephalomyocarditis virus in elephant sera from the Kruger National Park

Year	Sera positive/sera tested
1984	0/5
1985	0/23
1986	0/4
1987	1/8
1988	0/5
1989	2/8
1990	0/1
1991	1/5
1992	5/15
1993	62/116
1994	25/172

North region, and here antibody was present in 18 of 139 (13%) sera and nine of 16 herds, and in 18 of 73 (25%) elephants within seropositive herds (information on age and sex of individuals was not available). Only young calves, ≤ 7 years old, were tested from the Malelane area, and antibody occurred in seven of 33 (21%) sera and three of eight herds, and in seven of 13 (54%) sera within seropositive herds (in one herd five of five calves had antibody).

No significant EMC-antibody titres were detected in the radio-collared lioness and two sub-adult cubs bled at intervals, but the cub that died of bacterial pneumonia had a neutralizing titre of 128. Neutralizing antibody titres ranging from 8–64 were recorded in 19 of 23 sera from humans who had participated in elephant autopsies, and in none of 11 sera from humans who had only trapped and dissected rodents in the KNP or worked with EMC virus in the laboratory at NIV. The humans were bled in April 1994, and none of the seropositive persons could recall developing an illness after having participated in the elephant autopsies.

DISCUSSION

The present findings constitute strong evidence that EMC virus was responsible for the outbreak of mortalities in free-ranging African elephants observed in the KNP in 1993–1994: gross pathological changes compatible with the lesions known to be induced by EMC infection were seen in seven of eight elephants autopsied; characteristic histopathological lesions were found in the tissue of four of five elephants examined; EMC-virus antigen was demonstrated by immunocytochemistry in sections of heart muscle from two of three elephants; virus was isolated from the heart muscle of three of three elephants, and the presence of EMC-virus nucleic acid was demonstrated by RT-PCR in the heart muscle of two of three elephants. The failure to detect lesions or viral nucleic acid in the elephant killed for examination, which nevertheless yielded an isolation of the virus, is anomalous, but it is possible that virus titres and lesions were not yet maximal at the time that the animal was killed.

It is also possible that EMC-virus infection was responsible for at least some of the unexplained elephant deaths recorded from 1987 onwards, before the perceived outbreak of disease occurred at the end of 1993. Neutralizing antibody to the virus was

detected in elephant sera collected from 1987 onwards (Table 2), and was found in a single sample of rodent serum taken in the same year. It is notable that a respiratory-distress syndrome, with copious frothing at the nostrils and some deaths, was encountered in the course of elephant immobilization and capture operations from 1987 onwards (J.P. Raath & R.G. Bengis, unpublished observations 1981–1994). The problem was not experienced between 1981 and 1986, when the same immobilizing drugs and procedures were used, but occurred for the first time in 1987 and affected up to 10% of captured elephants by 1989.

The highest EMC-virus-neutralizing titre, 128, recorded prior to 1993, occurred in the serum of an elephant in which the respiratory syndrome was observed in 1989. The implication is that EMC infection may ostensibly cause subclinical disease which is exacerbated when elephants are acutely stressed. Furthermore, while it could be expected that mortalities associated with causes such as poaching and intraspecific fighting should affect mainly adult bulls, the predominance of bulls among the animals which died of unexplained causes, both before and during the perceived outbreak of disease in 1993–1994, may well be a feature of EMC-virus infection: female laboratory mice are more resistant to EMC disease than males, and the gender-related susceptibility can be reversed by the administration of testosterone or oestrogen (Zimmerman 1994). The results of the 1993 antibody survey indicate that the infection rate in female elephants was, if anything, slightly higher than in males, yet deaths were observed principally in bulls.

Although no accurate figure can be derived for the infection rate throughout the KNP, antibody prevalences suggest that up to 53% of elephants in the North may have undergone infection by June 1993, and that 13% of elephants in the Far North may have been infected by June 1994 (differences in prevalence of antibody may be related to underlying epidemiological differences between regions, or may merely reflect a difference in timing of the spread of infection). It can further be deduced from the results of the tests on sera collected from 1987 onwards, and from the distribution of carcases, that EMC infections had occurred in elephants in virtually all parts of the KNP by June 1994. If it is accepted that 13–53% of all elephants in the KNP had been infected by June 1994, that the population is approximately 7500, and that all of the 103 elephants which had died from unknown causes up to that date had succumbed to EMC infection, then it is possible to derive the rough estimate that, under natural conditions, the infection had a fatality rate of 2,6–10,6%. This calculation takes no account of the predilection of the disease for bull elephants, but at least it provides a rough indication of the losses to be expected in the extreme eventuality that all of the remaining susceptible elephants become infected.

The origins of the EMC infection and the exact timing of its introduction into the KNP must remain a matter of speculation. From 1954–1984, approximately 6000 rodents were tested for viruses in surveys conducted by the Arbovirus Unit at NIV, and the presence of EMC virus in the country was first demonstrated in 1961 when three isolations were made from *Mastomys* spp. (probably *M. coucha*) caught in the grounds of Rietfontein Hospital, Edenvale. Subsequently, 22 isolations were made from rodents, mainly *M. coucha*, caught in surveys in the Aliwal North and Prieska districts in 1968, implying that the virus was highly active during that year (NIV, unpublished records 1953–1994).

According to an experienced mammalogist (I.L. Rautenbach, personal communication 1994), 1968 was the last year in which there was a countrywide population explosion of rodents in South Africa analogous to that which apparently occurred in 1994, and the present serological results suggest that there was again widespread EMC-virus activity in 1994. The virus was also isolated in 1979, in association with an outbreak of disease in pigs in the KwaZulu-Natal midlands (Williams 1981). Hence, it has long been known that EMC virus is widely distributed in South Africa, and it must be conceded that the virus may well have been continuously present in the KNP for many years, or that it may have been present on previous occasions. However, no outbreak of disease in elephants similar to that described here is discernible in records kept since the late 1950s (V. de Vos, personal communication 1994).

Published information on the role of rodents in the dissemination of the infection is contradictory (Tesh & Wallace 1978; Zimmerman 1994). The EMC virus is similar to members of the *Enterovirus* genus of the *Picornaviridae* in that it causes gastro-intestinal infection and is capable of transmission by the faecal-oral route, and this has been demonstrated for rodents, including the African species, *M. natalensis* sensu lato (Kilham, Mason & Davies 1956).

Unlike the enteroviruses, EMC does not produce chronic intestinal infection, and excretion of the virus in the faeces of rodents may be limited to as short a period as 24 h after infection (Tesh & Wallace 1978). Partly for this reason, it was argued that rodents are not the prime source of infection in outbreaks of disease in other animals, and merely reflect the general circulation of the virus in multiple species in a given environment (Tesh & Wallace 1978).

Once EMC virus has been excreted in faeces, however, it displays a resistance to inactivation similar to that of the enteroviruses, and can cause persistent pollution of the environment, including standing water (Gainer, Sandefur & Bigler 1968). Hence, some authors have insisted that rodents play an important role in the faecal-oral spread of infection

to other animals. It was claimed, for instance, that rodent-control measures terminated a 9-month-long outbreak of EMC infection which killed 80 baboons in a breeding colony of 3060 individuals (Hubbard *et al.* 1992). In the KNP outbreak there is striking temporal correlation between the escalation of deaths in elephants, the population explosion of rodents, and the surge in prevalence of antibody in rodents (Fig. 2; Table 1). Moreover, large numbers of rodents were trapped in close proximity to artificial drinking troughs and water reservoirs. It is logical to conclude that rodents contributed significantly to the intensification of virus circulation in the environment, which led to the surge in fatalities of elephants, even if it cannot be proved that they were the original source of the infection. However, it is not known whether contagion or indirect transmission occurs between elephants.

It remains undetermined to what extent EMC infection contributed to the decline in myomorph-rodent populations noticed in the KNP after mid-1994, and whether or not the virus was responsible for the illness seen in squirrels. Myomorph rodents are susceptible to peripheral and oral infection (Zimmerman 1994) and presumably the virus must have killed a proportion of the population. The virus was suspected of having caused the deaths of large numbers of squirrels in Britain (Vizoso & Hay 1964; Vizoso, Vizoso & Hay 1964).

The failure to detect seroconversion in the three lions which fed on an elephant carcase in which lesions indicative of EMC infection were found, probably stems from an absence of the virus in the tissue consumed, since it is known that the virus disappears rapidly from most tissue and persists longest in the heart muscle of rodents (Tesh & Wallace 1978; Wee, Liu, Penn, Butany, McLaughlin, Sole & Liew 1992; Kyu *et al.* 1992), and virus could be isolated only from heart muscle in the tissue of elephants tested in the present study. Neutralizing antibody was detected in the serum of the lion cub found dead, and this animal may well have been predisposed to bacterial pneumonia through developing lung oedema as a result of EMC infection.

Presumably many other species were exposed to infection in the KNP, but carcases the size of large antelope and smaller, including those of primates, are scavenged rapidly and are less likely to be found than elephant carcases. Nevertheless, carcases of buffaloes, kudu antelope and even smaller animals are found readily when large numbers of the animals die during anthrax outbreaks or in the course of severe droughts, so it can be surmised that EMC infection did not produce heavy losses in species other than elephants, except possibly in small mammals such as rodents. The lack of a history of illness in any of the 19 members of the KNP staff in whom neutralizing antibody was found, lends support to the view that EMC virus has a low pathogenicity for humans.

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