Rabies in Red Foxes (*Vulpes vulpes*) Experimentally Infected with European Bat Lyssavirus Type 1

A. Vos¹, 8, T. Müller², L. Neubert¹, A. Zurbriggen³, C. Botteron³, D. Pöhle⁴, H. Schoon⁵, L. Haas⁶ and A. C. Jackson⁷

Addresses of authors: ¹Impstoffwerk Dessau Tornau (IDT) GmbH, 06855 Rossau, Germany; ²Institute of Epidemiology, WHO Collaborating Centre for Rabies Surveillance and Research, Federal Research Centre for Virus Diseases of Animals, 16868 Wusterhausen, Germany; ³NeuroCenter, Department of Clinical Veterinary Medicine, University of Bern, 3001 Bern, Switzerland; ⁴Regional Laboratory for Public Health and Veterinary Service – Saxony, 01099 Dresden, Germany; ⁵Institute for Veterinary Pathology, Veterinary Faculty, University of Leipzig, 04103 Leipzig, Germany; ⁶Institute of Virology, Hannover Veterinary School, 30559 Hannover, Germany; ⁷Departments of Medicine, and Microbiology and Immunology, Queen’s University, Kingston, ON K7L 3N6, Canada; ⁷Corresponding author: Tel.: +49 34901 885494; fax: +49 34901 885797; E-mail: ad.vos@idt-direct.de

Received for publication June 28, 2004

Summary

The susceptibility of red foxes (*Vulpes vulpes*) to European bat lyssavirus type 1 (EBLV-1) infection was examined. Eight foxes were inoculated intramuscularly (i.m.) with $10^{4.9}$ and $10^{5.1}$ foci-forming units (FFU) ($n = 4$) and 4 FFU ($n = 4$) and observed for up to 90 days. All foxes showed manifestations of a neurologic disorder (e.g. seizures, myoclonus, agitation), starting as early as 5 days post-infection (p.i.). Subsequently, all animals showed improvement followed by one or more relapses. One fox was killed 3 days after it recovered, 26 days post-infection. Two other foxes were killed 38 and 54 days post-infection after severe neurologic signs returned. All foxes developed a humoral immune response against EBLV-1 as determined in serum and brain tissues. However, no rabies virus antigen was detected in the brain, other tissues and secretions examined (e.g. salivary gland, saliva, tonsils, lungs) by using different standard diagnostic techniques [fluorescent antibody test, reverse transcription polymerase chain reaction (RT-PCR), rabies tissue culture inoculation test], with the exception of one fox in which EBLV-1 RNA was detected by RT-PCR in only the spinal cord. Brain tissues showed moderate to severe multifocal, mononuclear encephalomyelitis in the three foxes that were killed during the observation period, although no EBLV-1 virus was detectable in these tissues.

Introduction

The rabies situation in Europe has changed drastically in the last 25 years. As a result of oral vaccination campaigns of red foxes (*Vulpes vulpes*) against classical rabies virus (genotype 1 lyssavirus, RABV), terrestrial red foxes have been eradicated from large areas of Europe (Vos, 2003). Meanwhile, two other lyssaviruses have been identified, European bat lyssavirus types 1 and 2 (EBLV-1 and EBLV-2) (Bourhy et al., 1992). Although hundreds of EBLV-1 cases in bats have been reported from Europe (Müller, 2000; Fooks et al., 2003), spill-over infections in terrestrial mammals have been reported only rarely. So far, only two human cases have been reported from Russia and the Ukraine (Anonymous, 1986; Selimov et al., 1989). Furthermore, the virus has been isolated from several Danish sheep (*Ovis aries*) and a German stone marten (*Martes foina*) (Stougaard and Ammedrup, 1998; Müller et al., 2001a; Ronsholt, 2002). Although it is possible that such cases go largely undetected, it is generally assumed that spill-over infections are rare. As a result of oral vaccination campaigns against rabies in Europe, many virus isolates from rabies-positive animals have been collected and characterized. However, EBLV-1 was never detected in these animals. In Germany 304 135 foxes were examined between 1990 and 2003; EBLV-1 was detected in none of the 4845 rabies positive animals (W.W. Müller, unpublished results). Experimental susceptibility studies with EBLV-1 have been conducted in several terrestrial mammal species; mice (*Mus musculus*), dogs (*Canis familiaris*), cats (*Felis catus*) and ferrets (*Mustela putorius furo*) (Fekadu et al., 1988; Vos et al., 2004), but no information is available for the red fox, which is the major rabies vector species in Europe and is considered highly susceptible to rabies. This animal is also known to predate on bats (Bekker and Mostert, 1991), thus resulting in a risk of transmission of EBLV-1 infection. The purpose of this study was to evaluate the response of foxes to experimental EBLV-1 infection, and assess their potential role as a vector for this virus.

Materials and Methods

Animals

The captive-bred foxes were purchased from different commercial sources. On arrival, the foxes were kept for observation in individual wire cages with a connected nest-box at the outside animal enclosure (IDT). On the day of inoculation, the foxes were transferred to individual cages inside an isolation unit of the experimental animal facility. They were fed commercial food for minks (Frishfutter; Schirmer & Partner, Döhlen, Germany) and water was offered *ad libitum*. The foxes were inoculated and observed during two separate study periods: foxes 1–4 from 9 September to 3 December 2002 and foxes 5–8 from 30 July to 20 October 2003. The general appearance of the animals was recorded daily. Animals that showed severe signs of illness during the observation period...
after experimental infection were killed with Eunarcon (intracardial) after ketamine/xylocain sedation, and the complete carcasses of the animals were studied. The study was performed in compliance with the German Welfare Act of 1988.

**Virus**

The inoculation virus was isolated from a 10% brain suspension of a serotine bat (*Eptesicus serotinus*) from Wolkau (Germany) in July, 2000, and was identified as EBLV-1 using a panel of 10 anti-nucleocapsid monoclonal antibodies (Schneider, 1982). The virus material was adapted to murine neuroblastoma cells with four serial passages and diluted with minimal essential medium/serum neutralization test (MEM/SNT; $2.0 \times 10^6.0$ FFU/ml), Foxes 1–4 and foxes 5–8 received $10^7.06$ and $10^9.90$ FFU respectively. The virus was administered intramuscularly (i.m.) into the masseter muscles bilaterally.

**Sample collection**

The results obtained from foxes 1–4 led us to adapt the sampling scheme and sample preparation for the second study with foxes 5–8. Saliva was collected from the foxes on several fixed occasions (foxes 1–4: 7, 15, 21, 25, 37, 44, 58, 65, 71 and 85 days post-infection (p.i.) and foxes 5–8: 7, 14, 21, 28, 36, 54 and 90 days p.i.) by swabbing the oral cavity with a cotton swab. The cotton wool cylinder was placed in the holding tube that contained 2.0 ml MEM/SNT and a mixture of the following antibiotics: gentamicin (50 mg/l) and amphotericin B (2.5 mg/l). The tubes were stored at $-20^\circ$C until further examination by nested reverse transcription polymerase chain reaction (RT-PCR).

Blood was sampled on day 0, 7, 25, 58 and 85 p.i. (foxes 1–4) and day 0, 7, 14, 28, 54 and 90 p.i. (fox 5–8). If an animal showed severe clinical signs during the observation period, an additional blood sample was collected on the day of killing and serum was stored at $-20^\circ$C until further examination. After death, foxes 1–4 were stored frozen ($-20^\circ$C) and foxes 5–8 were shipped immediately to the laboratory. Upon arrival, the following tissues were fixed in formaldehyde or kept frozen for diagnostic evaluation: cerebral cortex, cerebellum, medulla, spinal cord, salivary glands (parotid and submandibular), tonsil, lung, liver, lymph nodes (cervical), spleen, kidney and heart. Brain tissues and spinal cord were examined for the presence of EBLV-1 antigen by standard techniques (see below). A histopathological examination of selected brain tissues was performed. The listed tissue samples and saliva were examined by nested RT-PCR, RT-PCR-positive samples were also examined by rabies tissue culture inoculation test (RTCIT).

**Assays**

**FAT and RTCIT**

The EBLV-1 viral antigen in the brain (cerebral cortex, cerebellum, medulla) and spinal cord of the inoculated animals was detected using the standard fluorescent antibody test (FAT) (Dean et al., 1996). For FAT-negative and FAT-suspect brain tissues virus isolation in neuroblastoma cell culture using the RTCIT was performed as described by Webster and Casey (1996). Generally, inoculated neuroblastoma cells were passaged at least three times in order to confirm a negative result.

**RT-PCR**

Prior to RNA extraction, tissue samples were homogenized with sterilized sea sand and resuspended in MEM. Total RNA was isolated from original samples (tissue or saliva) using RNAeasy Kit (Qiagen, Hilden, Germany). The total amount of RNA extracted was increased by conducting the elution step twice. The RT-PCR was conducted as nested RT-PCR (East et al., 2001), using the primer sets for first and second round RT-PCR, positive controls and the amplification protocol as described by Müller et al. (2004) and Vos et al. (2004). Carryover contamination and, hence, false-positive PCR results were avoided by strictly following the precautions for PCR as described by Kwok and Higuchi (1989).

**Histopathology**

Following post-mortem necropsy, pathological examinations were performed after staining formalin-fixed, paraffin-embedded tissues with haematoxylin–eosin. Brain tissues were also examined for changes of the myelin basic protein (MBP) using immunohistochemistry (IHC) and luxol fast blue staining (Zurbrüggen et al., 1984; Romeis, 1989).

**Serology**

Two methods were used for the detection of EBLV-1-specific antibodies in the blood samples and brain suspension of the foxes. An EBLV-1-adapted rapid fluorescent focus inhibition test (RFFIT) and immunoblot (IB) were used to detect virus neutralizing antibodies (VNA) and binding antibodies respectively (Vos et al., 2003, 2004). Prior to testing, sera were heat inactivated for 35 min at 56°C. To calculate the VNA titre, a 50% reduction in concentration of rabies virus in vitro was calculated by use of inverse interpolation (Müller et al., 2001b). For specificity reasons, the dilution with 100% reduction was used to calculate the VNA titre of the brain suspension. The starting working dilution of the brain tissues tested in RFFIT and IB was a 10% brain suspension (cerebral cortex, cerebellum and medulla) in MEM, as used for RTCIT. For EBLV-1 no reference material is available; hence the VNA titre cannot be converted into international units. The currently available international standard for rabies serology is based on genotype 1 (WHO/IABS, 1978).

**Results**

All eight foxes showed typical behavioural changes seen in rabies. The following signs were observed: apathy, paralysis of lower jaw, ataxia, muscle spasms, fatigue, tremor, agitation, myoclonus, aggression, debility, gasping and screaming. The occurrence, duration and intensity of these clinical signs differed among the animals. Fox 6 showed clinical signs at 5 days p.i., the other animals followed within the first 2 weeks p.i. The clinical course of the infection is shown in Fig. 1. Surprisingly, all animals showed spontaneous clinical improvement of their initial neurological signs. One animal (fox 5) was killed 3 days after recovery 26 days p.i. All but one animal
(fox 2) relapsed one or more times with clear clinical neurological signs during the remaining observation period. Due to the severity of the disease two foxes were killed (foxes 1 and 3) at 38 and 54 days p.i. respectively. None of the remaining animals showed clinical signs of rabies over the period from day 61 p.i. until the end of the observation period.

No EBLV-1-antigen was detected in any of the brain tissues examined. Attempts to reisolate the inoculated virus from brain tissues failed, indicating clearance of the virus. Also, all brain tissues and other tissues sampled tested negative by RT-PCR, with the exception of fox 5. Here, virus RNA was detected only in the cervical spinal cord by nested RT-PCR. This material tested negative by the less sensitive RTCIT. No virus or viral RNA was isolated from any of the oral swabs collected.

Histopathological examination revealed a moderate to severe non-suppurative, multifocal mononuclear polioencephalitis and meningitis in the three foxes killed during the observation period (Figs 2 and 3). In addition, fox 5 had a severe multifocal poliomyelitis. An indication of very mild perivascular non-suppurative encephalitis without involvement of the leptomeninges was observed only in fox 6 at the end of the observation period, although this animal did not show any clinical signs from day 34 p.i. onwards. None of the animals showed inclusion bodies. Luxol fast blue staining and IHC for MBP provided no evidence of a demyelinating process in the white matter in any of the brains (Fig. 4).

None of the foxes had detectable VNA titres to EBLV-1 prior to infection, but all developed VNA during the course of the infection (Fig. 5). The individual titres varied considerably during the observation period. The highest levels were observed approximately 1 month p.i. At the end of the observation period, all surviving animals still had high levels of VNA titres. Also, antibodies could be detected in the brains of five of the eight foxes (Table 1). The titres differed among the three brain tissues examined. The highest levels were observed in the medulla, followed by the cerebral cortex and the lowest titres were observed in the cerebellum.

**Discussion**

All foxes experimentally infected with EBLV-1 developed clinical neurological signs that are associated with rabies (Niezgoda et al., 2002). Histopathological examination of three animals revealed severe encephalitis, which was similar to the findings in a naturally infected German stone marten.
Individual titres; (—) average titre course.

The foxes that were not killed during the observation period after i.m. inoculation in the masseter muscle with a skunk RABV isolate (Hill and Beran, 1992). This is in contrast with our study, where the animals survived but showed neurological signs of rabies. Actually, the onset of the clinical signs in the foxes infected with EBLV-1, between 5 and 12 days p.i., was very rapid and may reflect direct spread of EBLV-1 into the CNS via peripheral nerves. It is still not fully understood what actually causes the clinical signs during rabies virus infection; neuronal dysfunction or death as a direct consequence of the virus infection or injury related to innate and adaptive immune responses (Shankar et al., 1992; Jackson, 2002).

Spontaneous recovery from rabies encephalitis after experimental infection of mice with a fox rabies virus isolate has been previously demonstrated (Jackson et al., 1989). However, in this case rabies virus antigen was found in CNS, one of the most commonly used criterion of a non-fatal rabies infection. The presence of rabies virus (neutralizing) antibodies in free-ranging wildlife as indicator of a non-fatal rabies infection has been described by many authors (Everard et al., 1981; Bigler et al., 1983; Rosatte and Gunson, 1984; Hill et al., 1992; Mebatsion et al., 1992; Gascoyne et al., 1993; Alexander et al., 1994; East et al., 2001). However, the demonstration of non-specific factors in sera has raised some doubts about the interpretation of the presence of VNA in some of these reports. Therefore, the presence of neutralizing antibody titres in sera is not sufficient evidence for recovery of a CNS infection. For example, the pathological and clinical signs observed could have been caused by an unknown adventitious agent. Actually, canine distemper virus infection was considered after preliminary examination of foxes 1 and 3. However, a more thorough examination of these animals and virus material (RT-PCR, in situ hybridization) ruled out this possibility (data not shown). A more definitive diagnostic test for demonstrating recovery from a rabies infection when no virus antigen can be detected is the presence of antibodies in the brain (Fekadu and Baer, 1980). In our study, five of the eight foxes had detectable levels of antibodies (neutralizing) antibodies in different parts of their brains, indicating that the virus had been present in CNS. Interestingly, VNA titres were higher in certain regions of the brain than in others and it remains to be clarified if the variation in observed VNA levels are a result of differences in infectivity in the brain tissues examined.

These results suggest that EBLV-1 was rapidly cleared from infected brain tissues, presumably around the time or shortly after the initial clinical illness. At 26 days p.i. EBLV-1 antigen could not be detected in the brain of fox 5. Dietzschold et al. (1992) demonstrated previously that antibodies can mediate rabies virus clearance from infected nervous tissue, thereby preventing death from a lethal rabies infection. However, in this case disruption of the blood–brain barrier is required (Hooper and Phares, 2003). Other immune effectors, like cytokines and neuropetides, may also contribute directly and indirectly to the clearance process by modulating immune responses and by inducing damage to the blood–brain barrier (Shankar et al., 1992; Hooper et al., 1998; Baloul and Lafon, 2003). The elimination of free virus and virus from infected cells may not always be associated with significant cell damage (Hooper et al., 1998). Furthermore, inflammatory changes present during early illness in association with clinical neurological signs could resolve by the end of the observation period after i.m. inoculation in the masseter muscle with a skunk RABV isolate (Hill and Beran, 1992). This is in contrast with our study, where the animals survived but showed neurological signs of rabies. Actually, the onset of the clinical signs in the foxes infected with EBLV-1, between 5 and 12 days p.i., was very rapid and may reflect direct spread of EBLV-1 into the CNS via peripheral nerves. It is still not fully understood what actually causes the clinical signs during rabies virus infection; neuronal dysfunction or death as a direct consequence of the virus infection or injury related to innate and adaptive immune responses (Shankar et al., 1992; Jackson, 2002).

Table 1. Results of the RFFIT and IB to detect virus neutralizing antibody titres in brain suspension of foxes experimentally infected with EBLV-1.

<table>
<thead>
<tr>
<th>Animal</th>
<th>Cerebellum</th>
<th>Cerebral cortex</th>
<th>Medulla</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>RFFIT</td>
<td>IB</td>
<td>RFFIT</td>
</tr>
<tr>
<td>1*</td>
<td>1 : 80 pos.</td>
<td>1 : 400 pos.</td>
<td>1 : 640 pos.</td>
</tr>
<tr>
<td>2</td>
<td>1 : &lt;10 neg.</td>
<td>1 : &lt;10 neg.</td>
<td>1 : &lt;10 neg.</td>
</tr>
<tr>
<td>3*</td>
<td>1 : 40 pos.</td>
<td>1 : 80 pos.</td>
<td>1 : 160 pos.</td>
</tr>
<tr>
<td>4</td>
<td>1 : 40 n.c.</td>
<td>1 : &lt;10 neg.</td>
<td>1 : &lt;10 n.c.</td>
</tr>
<tr>
<td>5*</td>
<td>1 : 40 pos.</td>
<td>1 : 80 pos.</td>
<td>1 : 80 pos.</td>
</tr>
<tr>
<td>6</td>
<td>1 : 80 pos.</td>
<td>1 : 80 pos.</td>
<td>1 : 200 pos.</td>
</tr>
<tr>
<td>7</td>
<td>1 : 20 pos.</td>
<td>1 : 20 pos.</td>
<td>1 : 160 pos.</td>
</tr>
<tr>
<td>8</td>
<td>1 : &lt;10 neg.</td>
<td>1 : &lt;10 neg.</td>
<td>1 : &lt;10 neg.</td>
</tr>
</tbody>
</table>

The samples were taken at the time of death. The results of RFFIT correspond to the dilution with a 100% reduction of the homologous test virus.

RFFIT, rapid fluorescent focus inhibition test; IB, immunoblot; pos., positive; neg., negative; n.c., not confirmative.

*Animals killed during the observation period.

(Müller et al., 2004). The foxes that were not killed during the observation period all improved and subsequently all but one fox had one or more relapses. However, no EBLV-1 RNA was detected in the central nervous system (CNS), non-neuronal tissues and saliva of the experimentally infected foxes with the highly sensitive nested RT-PCR assay, with the exception of one animal killed 26 days p.i. (fox 5) in which viral antigen was detected in the spinal cord. In comparison, brain tissues from all ferrets that developed marked clinical signs during the monophasic illness after experimental EBLV-1 infection using the same protocol (route of administration, virus material, assay methodology, housing conditions, etc.) tested positive for rabies (Vos et al., 2004). The observed difference between EBLV-1 infection in foxes and ferrets suggests a marked difference of neurovirulence in these two hosts. Fekadu et al. (1988) also observed a difference in virulence in EBLV-1 infection between cats and dogs after i.m. administration; cats died from rabies after experimental infection, but dogs did not show clinical signs and the infection was non-fatal. Also, raccoons (Procyon lotor) did not succumb or show clinical neurological signs associated with rabies during a 92-day observation period after i.m. inoculation in the masseter muscle with a skunk RABV isolate (Hill and Beran, 1992). This is in contrast with our study, where the animals survived but showed neurological signs of rabies. Actually, the onset of the clinical signs in the foxes infected with EBLV-1, between 5 and 12 days p.i., was very rapid and may reflect direct spread of EBLV-1 into the CNS via peripheral nerves. It is still not fully understood what actually causes the clinical signs during rabies virus infection; neuronal dysfunction or death as a direct consequence of the virus infection or injury related to innate and adaptive immune responses (Shankar et al., 1992; Jackson, 2002).

Spontaneous recovery from rabies encephalitis after experimental infection of mice with a fox rabies virus isolate has been previously demonstrated (Jackson et al., 1989). However, in this case rabies virus antigen was found in CNS, one of the most commonly used criterion of a non-fatal rabies infection. The presence of rabies virus (neutralizing) antibodies in free-ranging wildlife as indicator of a non-fatal rabies infection has been described by many authors (Everard et al., 1981; Bigler et al., 1983; Rosatte and Gunson, 1984; Hill et al., 1992; Mebatsion et al., 1992; Gascoyne et al., 1993; Alexander et al., 1994; East et al., 2001). However, the demonstration of non-specific factors in sera has raised some doubts about the interpretation of the presence of VNA in some of these reports. Therefore, the presence of neutralizing antibody titres in sera is not sufficient evidence for recovery of a CNS infection. For example, the pathological and clinical signs observed could have been caused by an unknown adventitious agent. Actually, canine distemper virus infection was considered after preliminary examination of foxes 1 and 3. However, a more thorough examination of these animals and virus material (RT-PCR, in situ hybridization) ruled out this possibility (data not shown). A more definitive diagnostic test for demonstrating recovery from a rabies infection when no virus antigen can be detected is the presence of antibodies in the brain (Fekadu and Baer, 1980). In our study, five of the eight foxes had detectable levels of antibodies (neutralizing) antibodies in different parts of their brains, indicating that the virus had been present in CNS. Interestingly, VNA titres were higher in certain regions of the brain than in others and it remains to be clarified if the variation in observed VNA levels are a result of differences in infectivity in the brain tissues examined.

These results suggest that EBLV-1 was rapidly cleared from infected brain tissues, presumably around the time or shortly after the initial clinical illness. At 26 days p.i. EBLV-1 antigen could not be detected in the brain of fox 5. Dietzschold et al. (1992) demonstrated previously that antibodies can mediate rabies virus clearance from infected nervous tissue, thereby preventing death from a lethal rabies infection. However, in this case disruption of the blood–brain barrier is required (Hooper and Phares, 2003). Other immune effectors, like cytokines and neuropetides, may also contribute directly and indirectly to the clearance process by modulating immune responses and by inducing damage to the blood–brain barrier (Shankar et al., 1992; Hooper et al., 1998; Baloul and Lafon, 2003). The elimination of free virus and virus from infected cells may not always be associated with significant cell damage (Hooper et al., 1998). Furthermore, inflammatory changes present during early illness in association with clinical neurological signs could resolve by the end of the observation period.
period. This could explain the fact that histopathological changes were observed in only four of eight foxes.

The abortive EBLV-1 infection in foxes is likely a consequence of the low pathogenicity of this virus for this species. The spread of apathogenic virus in the brain is less efficient than more pathogenic rabies viruses and may even become abortive (Dietzschold et al., 1985; Kuecera et al., 1985; Jackson, 2002). Reduced viral propagation enables the immune system to develop a specific response which subsequently leads to virus clearance from CNS (Coulon et al., 1998). The limited spread of the virus might be due to apoptosis and/or an apoptosis-driven enhanced immune response (Galelli et al., 2000; Pulmanausahakul et al., 2001). Morimoto et al. (1999) showed that the pathogenicity of rabies virus is inversely correlated with the capacity to induce apoptosis. Furthermore, low pathogenic rabies viruses induce a strong immune response in comparison with more virulent strains (Pulmanausahakul et al., 2001; Faber et al., 2002). We have not found any supporting evidence of an auto-immune encephalomyelitis which is characterized pathologically by perivascular demyelination, such as experimental allergic encephalitis (Prineas et al., 2002). In the foxes with a severe inflammation no demyelination could be observed with different special stains. This could be explained by the fact that the immune response was obviously not directed against any myelin or oligodendroglial antigen. Hence, the aetiology of the biphase or multiphasic clinical course observed in most of the animals remains obscure, but it also appears unrelated to permissive EBLV-1 infection. Because the virus was rapidly cleared from the brain of the infected foxes, a carrier state can be excluded. The results of this study reveal that EBLV-1 infections in foxes could frequently go undetected when the animals are submitted for routine rabies diagnosis. Additional serological studies would be needed to indicate EBLV-1 infection when the animals are examined for rabies provided that naturally EBLV-1 infected animals would seroconvert above detectable levels. Of course, in rabies endemic areas with RABV or in areas where oral rabies vaccine baits are, or recently have been distributed, this method could not be used. Furthermore, the results of the current study suggest that foxes are very unlikely to serve as a transmitting host for EBLV-1, because no virus could be found in the salivary glands and saliva during the entire observation period. However, we should keep in mind that the same virus isolate was used in the ferrets and foxes. Davies (2003) reported that mice inoculated with two different isolates from two Eptesicus fuscus caused different levels of morbidity and mortality; one isolate was non-pathogenic, while most mice inoculated with the second isolate became sick and consequently died. This needs to be verified for EBLV-1 as well. However, it must be kept in mind that the results obtained during these experimental studies could be a result of a relatively high inoculation dose. Under natural conditions, the amount of virus particles transmitted could be much lower, resulting in an abortive and or subclinical infection.

Acknowledgements
We are grateful to the contributions of Jeanette Burow, Astrid Schametat, Uli Wulle and Simone Becker for their work in the laboratory and looking after the animals. Furthermore, we thank Jim Cox for the preparation of the inoculation material.

References


