Report of isolations of unusual lyssaviruses (rabies and Mokola virus) identified retrospectively from Zimbabwe

J Bingham, S Javangwe, C T Sabet, A Wandeler, L H Nel

ABSTRACT

Rabies isolates that had been stored between 1983 and 1997 were examined with a panel of anti-lyssavirus nucleocapsid monoclonal antibodies. Out of 56 isolates from cats and various wild carnivore species, 1 isolate of Mokola virus and 5 other non-typical rabies viruses were identified. The Mokola virus isolate was diagnosed as rabies in 1993 from a cat. Genetic analysis of this isolate suggests that it falls in a distinct subgroup of the Mokola virus genotype. The 5 non-typical rabies viruses were isolated from honey badgers (Mellivora capensis), African civets (Civettictis civetta) and an unidentified mongoose (Herpestidae). These isolates are representatives of rarely-reported wildlife-associated strains of rabies, probably maintained by the slender mongoose (Galera sanguinea). These findings indicate that both Mokola virus and the mongoose-associated variant may be more common in Zimbabwe than is apparent from routine surveillance.

Key words: lyssavirus, Mokola virus, rabies, Zimbabwe.

lyssaviruses maintained by canid species in southern Africa. Five isolates produced a different reaction pattern: 2 from African civets (CVL reference 19671 from Rusape in 1991, 21179 from Penhalonga 1992), 2 from honey badgers (19571 from Gweru 1991, 20948 from Bulawayo 1992) and 1 from an unidentified mongoose (22107 from Rusape 1994). Subsequent gene sequence analysis confirmed that these isolates are related to herpestid-associated rabies (genotype 1) variants that cycle in southern Africa (data not shown).

Rabies viruses associated with slender mongooses (Genetta sanguinea) were first recognised as being different after several isolates from the 1970s were examined with monoclonal antibody panels. Since then, only 2 other isolates suspected of belonging to this group were identified, 1 in a slender mongoose from Fort Rixon, east of Bulawayo, in 1991 (CVL rabies number 19518) and 1 in an African civet from Wedza in 1994 (number 22574). Finding other isolates belonging to this group indicates that it may be more prevalent in Zimbabwe than initially suspected. Surprisingly, most of the recent isolates were not obtained from slender mongooses, raising the possibility that other species may participate in the maintenance of the virus variant.

One isolate gave the reaction pattern characteristic of Mokola virus (CVL rabies number 21846). This isolate had been made in November 1993 from a domestic cat on a farm close to the town of Selous, about 70 km southwest of Harare. According to the case submission form the male cat had returned home, after having disappeared for some days, with cuts and bruises around the head and back. On presentation to the private veterinarian who submitted the case, its body temperature was subnormal and its reflexes were depressed. No aggression was reported and no rabies vaccination history was recorded. The cat was euthanised in extremis and the carcass was submitted to the Central Veterinary Laboratory, Harare, for rabies diagnosis. Direct fluorescent antibody and mouse inoculation tests were both positive for rabies.

The identification of this isolate is further evidence that Mokola virus is widely prevalent, although rarely detected, in Africa. Most other isolates in southern Africa, including 1 from Ethiopia, have been found in domestic cats, indicating that they are a major indicator species for the virus. However, the epidemiology of Mokola virus infection is poorly understood: the maintenance host is not known, but obviously transmits the virus to domestic cats more readily than to other commonly-tested species. Mokola virus infection is a potentially serious zoonotic disease. It may cause fatal disease in humans and current rabies vaccines do not appear to protect against it. Infected animals, particularly cats, present signs that may be confused with rabies and other neurological conditions. Pet owners, veterinarians and laboratory personnel should be particularly aware of the zoonotic dangers of this virus.

In order to establish the relationships of this virus with other Mokola virus isolates and other local lyssaviruses, part of the nucleoprotein gene of the isolate was sequenced as described previously. Figure 1 shows a phylogenetic tree that was derived from an alignment of the N1–N2 nucleotide sequences of various southern African lyssavirus isolates. This interpretation places the isolate within genotype 3 (Mokola virus) of the lyssaviruses, although it is clearly distinguishable from other isolates in this genotype.

Monoclonal antibodies against nucleoprotein and phosphoprotein epitopes clearly discriminate among at least 5 groups of Mokola virus variants: the West African prototype, 3 distinct South African variants, and the Zimbabwean isolate (Table 2). These data, together with those summarised in Fig. 1, indicate that there is considerable diversity within lyssavirus genotype 3 viruses and that separate groups within this genotype appear to be associated with different geographical regions. The isolate described here originated from an area that is over 300 km from the nearest other area from which Mokola virus has been isolated, namely Bulawayo, from cats in the early 1990s.

The genetic and antigenic diversity of Mokola virus in the relatively small geographical range of southern Africa may indicate long periods of evolution, adaptation to local ecological conditions and different host species, and/or reduced constraints on genetic variation. The magnitude of variation in epitopes

<table>
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<th>Monoclonal antibody</th>
<th>SDF12</th>
<th>24FF11</th>
<th>26AB7</th>
<th>32HD2</th>
<th>M853</th>
<th>M856</th>
<th>M1001</th>
<th>M1005</th>
<th>M1336</th>
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<td></td>
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<td>Rabies (mongoose)</td>
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<td>Mokola virus</td>
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*Includes 24 domestic cats (*Felis domestica*), 5 wild cats (*Felis lybica*), 8 honey badgers (*Mellivora capensis*), 8 African civets (*Civettictis civetta*), 3 unspecified mongooses (*Herpestidae*) and 2 genets (*Genetta* sp.)*

*Includes 2 honey badgers, 2 African civets and 1 unspecified mongoose.*

Table 1: Monoclonal antibody profiles for 56 Zimbabwean lyssavirus isolates passaged through mice. Black cells indicate a positive reaction; white cells, a negative reaction; grey cell, a variable (negative or weak positive) reaction.

recognised by antibodies may also reflect functional diversity. Further surveillance for identification of unusual lyssaviruses, determination of their reservoir species and further genetic and antigenic characterisation of new isolates will enable us to gain a better understanding of the epidemiology of these unusual lyssaviruses.

REFERENCES


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Table 2: Monoclonal antibody profiles for Mokola viruses as determined by indirect immunofluorescent tests carried out in mouse neuroblastoma cell cultures. Apart from the new isolate from Zimbabwe (21846) the isolates tested include the Mokola virus prototype from Nigeria and South African isolates from Pinetown and Pietermaritzburg (97/252, 97/229 and 98/071), from Umlhlanga Rocks (700/70) and from East London (543/95, 112/96 and 322/96). Black cells indicate a positive reaction and white cells indicate a negative reaction. Target proteins are the nucleoprotein (N) and the phosphoprotein (P).

<table>
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<tr>
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