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

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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 (*Galerella 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.

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The lyssaviruses are classified as a genus within the family Rhabdoviridae and include classical rabies virus (genotype 1) and a number of other rare genotypes, represented by Lagos bat, Mokola and Duvenhage viruses from Africa and the European and Australian bat lyssaviruses[2,9]. In southern Africa, distinct rabies virus variants are maintained by canid species, both domestic and wild, and by Herpestidae (mongoose) species[11,19]. The maintenance host of Mokola virus (genotype 3) is not known. Most isolates have been made from domestic cats, although isolates have also been made from shrews (*Crocidura* sp.), a rodent *Lophuromys sikapusi,* a domestic dog and human beings[3,4,6,7,10,12–14,17,18,20]. Mokola virus has only been detected in Africa and appears to occur throughout the continent, as it has been isolated from West Africa, Central Africa, Ethiopia, Zimbabwe and South Africa. Mokola virus has been identified by surveys or in those areas where methods appropriate to identifying genotypes other than classical rabies virus were employed[20]. Lyssaviruses cause clinical disease similar to that caused by classical rabies virus. The virus groups also have some antigenic similarity. Therefore, in many laboratories unusual lyssavirus infection is indistinguishable from classical rabies by direct fluorescent antibody tests, because the conjugates used react with viruses of more than 1 genotype. Many of the cases reported in previous studies were initially submitted for rabies diagnosis and some were initially diagnosed as rabies.

This report describes a survey in which a selection of viruses isolated from cases of neurological disease and initially diagnosed as rabies were typed using monoclonal antibodies. The isolates were collected between 1983 and 1997 from various areas throughout Zimbabwe and were diagnosed as rabies by the fluorescent antibody test at the Central Veterinary Laboratory in Harare. They were passaged once in mice and the mouse brain material stored in liquid nitrogen. Fifty-six isolates, selected from unusual hosts including domestic and wild cats (*Felis* sp.), African civets (*Civettictis civetta*), honey badgers (*Mellivora capensis*), genets (*Genetta genetta*) and various mongoose species (Herpestidae) were typed. Typing was carried out at the Rabies Unit, Onderstepoort Veterinary Institute, using a panel of anti-lyssavirus nucleocapsid monoclonal antibodies produced in mice (by the Centre of Expertise for Rabies, Canadian Food Inspection Agency). Further antigenic characterisation of Mokola virus isolates using larger monoclonal antibody panels on isolates adapted to mouse neuroblastoma cell cultures was done at the Canadian Center of Expertise for Rabies as described elsewhere[15].

The test used for monoclonal antibody typing was an indirect immunofluorescent antibody test conducted on infected mouse brain. Occasionally the mice inoculated with particular virus isolates mount a significant immune response to the virus. Such autogenous antibodies leaking into the brain tissue *post mortem* can simulate positive results in indirect immunofluorescent tests. To check for the presence of autogenous antibody, smears of the 1st passage mouse brain were made on multi-well slides and stained directly with fluorescein-labelled anti-mouse anti-globulin. The smears were read under ultra-violet microscopy. Second passage brain material was prepared when the first passage material showed significant autogenous antibody as indicated by positive staining. Once suitable brain material had been identified, smears were made on teflon-coated multi-well slides. The smears were fixed in cold acetone for 10 minutes and then air-dried. Twelve microlitres of each mouse monoclonal antibody was placed into the wells according to a predetermined scheme. After 30 minutes of incubation at 37 °C the slides were washed separately in phosphate-buffered saline (PBS, pH 7.3) and blot-dried. Fluorescein-labelled anti-mouse conjugate was then applied to each well, the slides were incubated for a further 30 minutes, washed in PBS, blot-dried and mounted under a cover-slip with 50 % glycerol-saline. The smears were read by ultra-violet microscopy. The fluorescence of each well was scored as positive (on a scale of 1–3) or negative.

Table 1 shows the reaction patterns of the isolates studied, using mouse brain material. Most isolates exhibited the typical pattern found for genotype 1

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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.**

Monoclonal antibody	5DF12	24FF11	26AB7	32HD2	M853	M856	M1001	M1005	M1336	M1407	Biotype
50 isolates[a]	■	■	■	■	■	■			■	■	Rabies (canid)
5 isolates[b]	■	■	■	■		■				■	Rabies (mongoose)
Cat 21846							■	■			Mokola virus

[a]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.).
[b]Includes 2 honey badgers, 2 African civets and 1 unspecified mongoose.

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 (*Galerella sanguinea*) were first recognised as being different after several isolates from the 1970s were examined with monoclonal antibody panels[8,21]. 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 euthanased *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[6,7,13,20] 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[4] and current rabies vaccines do not appear to protect against it[1,5,20]. 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[16]. Figure 1 shows a phylogenetic tree that was derived from an alignment of the N1–N2 nucleotide sequences of various southern African lyssavirus isolates[16]. 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 1980s[6,7].

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

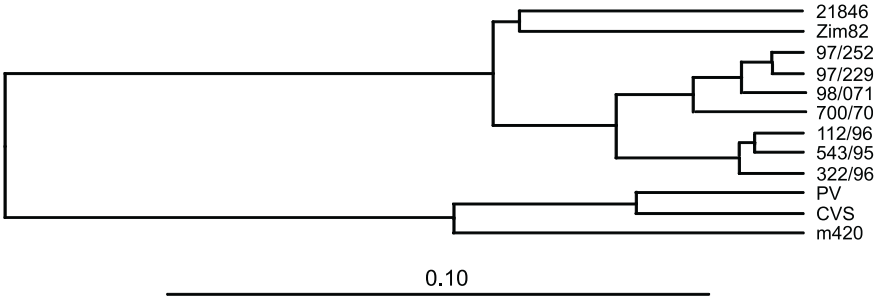

Fig. 1: **Phylogenetic reconstruction indicating the relationship of the new Mokola isolate from Zimbabwe** (21846/93) **with another isolate from Zimbabwe** (Zim 82[6,7]) **and with 7 South African isolates of the virus** (see Table 2 for explanation)**. The rabies virus genotype is represented by the Pasteur virus** (PV)**, the challenge virus standard** (CVS) **and a mongoose virus isolate from South Africa** (m420)**. The inferred phylogeny is based on sequence alignment of the N1–N2 nucleoprotein gene sequences as previously described[16].**

Table 2: **Monoclonal antibody profiles for Mokola viruses as determined by indirect immunofluorescent tests carried out in mouse neuroblastoma cell cultures[15]. 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)[21]**, from Umhlanga Rocks** (700/70)[14] **and from East London** (543/95, 112/96 and 322/96)[14,21]**. Black cells indicate a positive reaction and white cells indicate a negative reaction. Target proteins are the nucleoprotein** (N) **and the phosphoprotein** (P)**.**

Monoclonal antibody	20HF8 M879	11DG10	M1335 M1336	M863 M874	M867 M873	M878	M862 M882 M1015 M1025 M1027	M1700	M1718
Target protein	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**P**	**P**
Prototype			■		■		■		■
21846				■	■		■		
97/252	■			■	■	■		■	■
97/229	■			■	■	■		■	■
98/071	■			■	■	■		■	■
700/70				■	■	■	■	■	■
543/95		■	■	■	■	■	■	■	■
112/96		■	■	■	■	■	■	■	■
322/96		■	■	■	■	■	■	■	■

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.

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