RESEARCH COMMUNICATION

Importation of canid rabies in a horse relocated from Zimbabwe to South Africa

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ABSTRACT

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In July 2003 a 2-year-old Thoroughbred colt was imported from Harare, Zimbabwe to the Ashburton Training Centre, Pietermaritzburg, South Africa. Five months after importation, the colt presented with clinical signs suggestive of rabies: it was uncoordinated, showed muscle tremors and was biting at itself. Brain tissue was submitted for analysis and the clinical diagnosis was confirmed by the fluorescent antibody test and reverse-transcription polymerase chain reaction (RT-PCR). Phylogenetic analysis of the nucleotide sequence of the cytoplasmic domain of the glycoprotein and the G-L intergenic region of the rabies virus confirmed it to be an infection with a canid rabies virus, originating from an area in Zimbabwe endemic for the domestic dog (*Canis familiaris*) and side-striped jackal (*Canis adustus*) rabies.

Keywords: Canid rabies biotype, epidemiology, lyssavirus, rabies virus, South Africa, Zimbabwe

INTRODUCTION

Rabies virus (RV) is the prototype member of the *Lyssavirus* genus*,* Rhabdoviridae family, of the order Mononegavirales (Wunner, Larson, Dietzschold & Smith 1988; Tordo & Kouknetzoff 1993). Rabies virus possesses a non-segmented, negative-stranded RNA genome of ~12 kb in length. The viral genome contains information for five proteins: nucleoprotein (N), matrix protein (M), phosphoprotein (P), glycoprotein (G) and polymerase (L).

Epidemiologically, RV is found virtually worldwide and is perpetuated in several domestic and wild carnivore species. In the southern African countries of Zimbabwe and South Africa, rabies virus exists as two epidemiologically separate lineages (referred to as canid and mongoose rabies biotypes), which has been confirmed by antigenic and genetic studies (Foggin 1988; King, Meredith & Thomson 1993, 1994; Von Teichman, Thomson, Meredith & Nel 1995; Nel, Jacobs, Jaftha & Meredith 1997). Prior to the era of antigenic and genetic tools for studying the epidemiology of rabies, the existence of the two rabies biotypes was postulated from historical records and case surveillance data.

In this paper, we demonstrate the use of reverse transcription polymerase chain reaction (RT-PCR) for confirming a clinical diagnosis of rabies infection in a 2-year-old Thoroughbred colt imported in July 2003, from Golden Acres, Harare in Zimbabwe to the Ashburton Training Centre, South Africa (see Fig. 1 for approximate locations of the two sites).

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FIG. 1 Map of Zimbabwe and South Africa showing the approximate geographical locations of Harare (where the 2-year-old colt originated) and Pietermaritzburg (where it subsequently developed clinical signs compatible with rabies)

TABLE 1 Virus isolates included in the phylogenetic comparison

Lab. ref no.	Species	Locality and country of origin	Year isolated	Accession no.
22642[a]	Jackal (*C. adustus*)[b]	Glendale, Zimbabwe	1994	AF177088
21467	Canine[c]	Goromonzi, Zimbabwe	1993	AF177066
21111	Jackal (*C. adustus*)	Bromley, Zimbabwe	1992	AF177083
19649	Jackal (*C. adustus*)	Marondera, Zimbabwe	1991	AY605006
28498	Jackal (*C. adustus*)	Marondera, Zimbabwe	2002	AY605041
23357	Jackal (*C. adustus*)	Wedza, Zimbabwe	1995	AF177090
24465	Canine	Wedza CL[d], Zimbabwe	1996	AF177074
23367	Jackal (*C. adustus*)	Marondera, Zimbabwe	1995	AF177093
24299	Canine	Musikavanhu CL, Zimbabwe	1996	AF177073
23652	Canine	Mutare, Zimbabwe	1995	AF177071
A03/646[a]	Equine	Pietermaritzburg, South Africa	2003	AY605005
29263	Lion (*Panthera leo)*	Marondera, Zimbabwe	2003	AY605014
24505	Canine	Gutu, Zimbabwe	1996	AF177075
16387	Canine	Zhombe CL, Zimbabwe	1986	AF177057
16254	Canine	Odzi, Zimbabwe	1986	AF177055
22547	Canine	Kumutsenzere, Zimbabwe	1994	AF177070
16347	Canine	Troutbeck, Nyanga, Zimbabwe	1986	AF177056
21869	Canine	Nyakasoro, Pfungwe CL, Zimbabwe	1993	AF177069
21057	Canine	Muzarabani CL, Zimbabwe	1992	AF177064
20519	Canine	Lower Gweru, Zimbabwe	1992	AF177060
A00/413	Canine	Pietermaritzburg, South Africa	2000	AY605042
A90/57	Canine	Durban, South Africa	1990	AF177101
A90/352	Canine	Durban, South Africa	1990	AF177100
A95/755	Canine	Amanzimtoti, South Africa	1995	AF303081
28522	Jackal (*C. mesomelas*)	Gwanda, Zimbabwe	2002	AY605038
23374	Jackal (*C. mesomelas*)	Bulawayo, Zimbabwe	1995	AF177091
21579	Jackal (*C. mesomelas*)	Tsholotsho CL, Zimbabwe	1993	AF177086
17722	Jackal (*C. mesomelas*)	Gwanda, Zimbabwe	1988	AF177079
20034	Canine	Bvumba, Mutare, Zimbabwe	1991	AF177059
16838	Canine	Shangani, Zimbabwe	1987	AF177058
27792	Jackal (*C. mesomelas*)	Fort Rixon, Zimbabwe	2001	AY605032
27890	Jackal (*C. mesomelas*)	Insiza, Zimbabwe	2001	AY605039
19671	*Civettictis civetta*	Rusape, Zimbabwe	1991	AF304188

[a] Laboratory reference numbers: Isolates from Zimbabwe use the Harare Central Veterinary Laboratory rabies reference; isolates prefixed by "A" indicate the Allerton Provincial Veterinary Laboratory reference numbers, Pietermaritzburg

[b] Where the jackal species was not definitively identified at collection, the species zone that it originated in, and hence the principal host, is given in parenthesis (see Bingham *et al.* 1999b)

[c] We use the term "canine" to refer to the domestic dog, *Canis familiaris*

[d] CL stands for communal lands

The colt presented with clinical signs suggestive of rabies on 6 December 2003. It was uncoordinated, manifested muscle tremors and was biting at itself. It was subsequently admitted to the Summerfield Equine Hospital on the same day where it was examined by a veterinary surgeon. It was euthanased the following day and brain tissue was submitted for rabies testing to the Allerton Provincial Laboratory in Pietermaritzburg, KwaZulu-Natal. Although there was no recorded history of a dog bite and no skin wounds were detected, the brain sample tested positive by the fluorescent antibody test (FAT). The rabies virus (laboratory reference no. A03/646) was then subjected to molecular characterization in order to establish its origin.

MATERIALS AND METHODS

For molecular characterisation of the rabies virus (A03/646), it was decided to target the variable glycoprotein gene. RT-PCR and DNA sequence analyses were carried out at the Rabies Unit, Onderstepoort Veterinary Institute, Pretoria as described previously (Von Teichman *et al*. 1995; Sabeta, Bingham & Nel 2003). An amplicon of the expected size (~850 bp) was obtained with the G/L primer set and purified with a Wizard PCR CleanUp System (Promega, USA). An aliquot (~50 ng) of the purified amplicon was sequenced using the BigDye Terminator system (Perkin Elmer) with the same primer pair (G/L) as in the previous RT-PCR steps. A consensus sequence of the equine rabies virus was assembled after comparison of the forward and reverse sequences with Sequence Navigator Software (PE Applied Biosytems).

Phylogenetic analysis of the nucleotide sequence of the rabies virus together with nucleotide sequences of other virus isolates from our database (Table 1) was done using the Phylip software package (Phylogeny Inference Package).

In brief, a multiple alignment of a 592-nucleotide region of the nucleotide sequences included in the analysis was generated with ClustalW (Thompson, Higgins & Thompson 1994). Distance calculations were done using the Kimura 2-parameter model for evolutionary rate (Kimura 1980). For construction of the phylogenetic trees, the Neighbour Joining (NJ) method combined with a 1 000 bootstrap iterations was used (Saitou & Nei 1987; Hills & Bull 1993). The program TREEVIEW was used to display the graphical output of the tree (Fig. 2) (Page 1996).

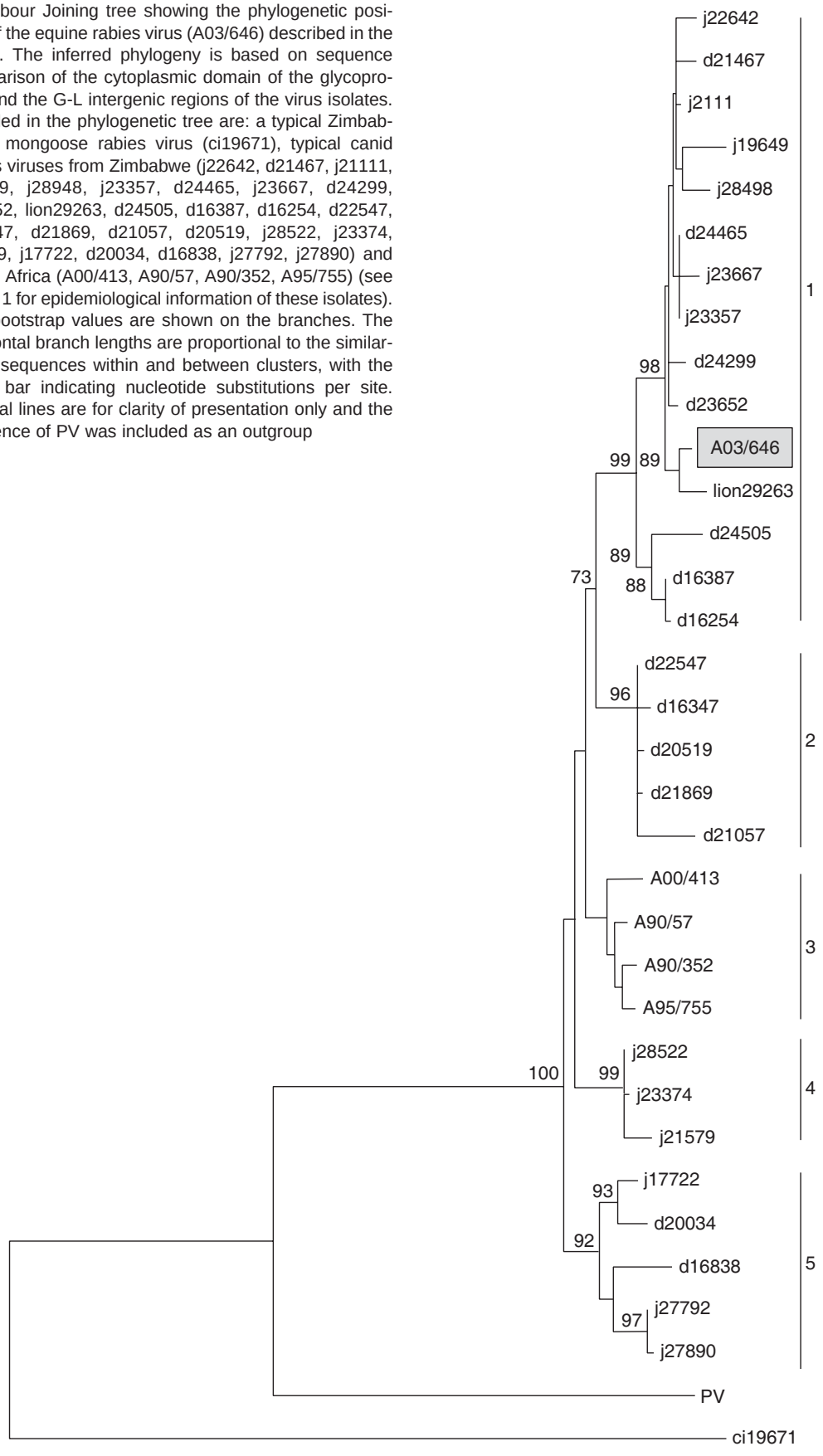
RESULTS AND DISCUSSION

Both the direct immunofluorescent antibody test (FAT) and RT-PCR confirmed the rabies infection. The rabies virus was successfully amplified by the G/L primer pair to yield an expected product of approximately 850 bp (not shown). Nucleotide sequencing of the purified PCR amplicon and phylogenetic comparison with other nucleotide sequences from our database of Zimbabwean and South African rabies G-L nucleotide sequences demonstrated it to be a canid rabies virus (genotype 1) originating from Zimbabwe (see Fig. 2).

Despite the close genetic relatedness of the canid rabies virus isolates compared here [mean sequence homology of 96.3 % calculated in MEGA (Kumar, Tamura, Jakobsen & Nei 2001)], the rabies virus (A03/646) was found to cluster with Zimbabwean canid rabies virus isolates (Cluster 1) endemic within the domestic dog (*Canis familiaris*) and side-striped jackal (*Canis adustus*) (Bingham, Foggin, Wandeler & Hill 1999a; Bingham, Foggin, Wandeler & Hill 1999b; Sabeta *et al*. 2003). Cluster 1 was statistically supported with a bootstrap value of 98 %. Cluster 2 is composed of virus isolates exclusively from domestic dogs in north-eastern Zimbabwe. Clusters 1 and 2 consist of Zimbabwean viral isolates and can be distinguished from isolates from domestic dogs in KwaZulu-Natal (cluster 3), the region in which the colt developed clinical rabies 5 months after it had been imported from Harare. Cluster 4 is made up of viruses exclusively from black-backed jackal (*Canis mesomelas*) and cluster 5 from both domestic dogs and *C. mesomelas*. Both clusters 4 and 5 represent viral isolates that are associated with rabies cycles in the *C. mesomelas*-zone of southern Zimbabwe and northern South Africa (Sabeta *et al*. 2003).

Rabies in horses is a relatively uncommon disease and transmission of the disease from horses to humans is rare (Green, Smith, Vernau & Beacock 1992). However, a potential risk to veterinarians, horse handlers and horse owners does exist and should be emphasized. Given the high incidence of rabies in Zimbabwe and in the sub-region as a whole, it would be advisable for horse owners and those in the horse industry alike to vaccinate their animals against rabies. The absence of wounds in the 2-year-old colt does not exclude inoculation by a bite from a rabid animal, because had bite wounds been present, they might have been very small and puncture-like and were therefore not detected, or they could have healed prior to the development of clinical signs. The rabies virus isolate investigated

FIG. 2 Neighbour Joining tree showing the phylogenetic position of the equine rabies virus (A03/646) described in the paper. The inferred phylogeny is based on sequence comparison of the cytoplasmic domain of the glycoprotein and the G-L intergenic regions of the virus isolates. Included in the phylogenetic tree are: a typical Zimbabwean mongoose rabies virus (ci19671), typical canid rabies viruses from Zimbabwe (j22642, d21467, j21111, j19649, j28948, j23357, d24465, j23667, d24299, d23652, lion29263, d24505, d16387, d16254, d22547, d16347, d21869, d21057, d20519, j28522, j23374, j21579, j17722, d20034, d16838, j27792, j27890) and South Africa (A00/413, A90/57, A90/352, A95/755) (see Table 1 for epidemiological information of these isolates). The bootstrap values are shown on the branches. The horizontal branch lengths are proportional to the similarity of sequences within and between clusters, with the scale bar indicating nucleotide substitutions per site. Vertical lines are for clarity of presentation only and the sequence of PV was included as an outgroup



and described here would most likely have been transmitted as a result of a dog bite. This is highly probable considering that the canid rabies variant is widespread and prevalent, making it the most threatening variant for humans and domestic carnivore species alike (Bingham *et al*. 1999a). This calls for stricter import/export regulations for animals in transit in order to diminish the spread of rabies across regional borders.

Although horses are known to be moderately susceptible to rabies infection, long incubation periods of up to 5 months, as shown in this case, may be unusual. The clinical signs of rabies are variable in horses and generally appear after an incubation period of 2–6 weeks, although in some it may be up to 3 months (Beran 1981). However, rabies virus has been isolated from the saliva of a dog that survived 20 months post-exposure to the virus (Green *et al*. 1992). Although it has been shown that rabies virus remain at the site of a bite for most of the incubation period, which is generally 10–90 days, longer incubation periods have been described by Smith, Fishbean, Rupprecht & Clark (1991).

This investigation illustrates the usefulness of RT-PCR as a tool for studying animal viral diseases such as rabies. Furthermore, it increases our understanding of epidemiological relationships of lyssaviruses not only through establishing their genetic relationships but also by providing us with information on the origin, geographical distribution and paths of transmission of the various strains of the virus (Brown 1994).

Several molecular epidemiological studies of rabies have been conducted in the southern African subregion (Nel, Thomson & Von Teichman 1993; Von Teichman *et al*. 1995; Nel *et al*. 1997; Sabeta *et al*. 2003). Further molecular studies of lyssaviruses in countries in the sub-region that include Botswana, Namibia, Mozambique, Swaziland, Lesotho and Zambia should be encouraged, and are already being realized. These studies are essential in order to elucidate the dynamics of rabies in the sub-region. An expansion of our current nucleotide sequence database of rabies viruses would thus be useful for tracing the routes of infection as illustrated by the equine case described here and also for establishing concrete measures to control the disease.

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