Epidemiology of human rabies in South Africa, 1983-2007

Jacqueline Weyer[a*], Ania V. Szmyd-Potapczuk[c*], Lucille H. Blumberg[a,b], Patricia A. Leman[a], Wanda Markotter[c], Robert Swanepoel[a], Janusz T. Paweska[a] , Louis H. Nel[c]

[a] Special Pathogens and [b]Outbreak Response Units, National Institute for Communicable Diseases of the National Health Laboratory Service, Private Bag X4, Sandringham, 2131, South Africa

[c] Department of Microbiology and Plant Pathology, University of Pretoria, 0002, South Africa

* Authors contributed equally

Corresponding author:
Jacqueline Weyer
Telephone: +27 (0) 11 386 6376
Fax: +27 (0) 11 882 3741
Email: jacquelinew@nicd.ac.za
Postal address: Special Pathogens Unit
National Institute for Communicable Diseases of the National Health Laboratory Service
Private Bag X4
Sandringham, 2131
South Africa

Ania V. Szmyd-Potapczuk s22003322@tuks.co.za
Lucille H. Blumberg: lucilleb@nicd.ac.za
Patricia A. Leman: patl@nicd.ac.za
Wanda Markotter: wanda.markotter@up.ac.za
Robert Swanepoel: bobs@nicd.ac.za
Janusz T. Paweska januszp@nicd.ac.za
Louis H. Nel: louis.nel@up.ac.za

Abstract

Rabies remains a global public health problem but increasingly so in the developing world. Given a lack of awareness, priority and diagnostic

capability, very few developing countries, especially in Africa, report on laboratory confirmed human rabies cases. Here we present a retrospective study on the epidemiology of human rabies in Republic of South Africa for a 25 year period, 1983-2007, based on laboratory confirmed cases. The study highlights the role of the domestic dog as a reservoir and vector of rabies and contrasts this to the almost negligible contribution of wildlife vectors to the overall burden of human rabies in dog rabies endemic areas. From the collective data set, epidemiological aspects that include various features of these human rabies cases as well as failures in or towards the treatment of exposures are reported.
Molecular analysis of virus isolates did not identify any additional cases of rabies attributed to infection with the Duvenhage, Lagos bat or Mokola or any other rabies-related viruses.

Keywords: human rabies, South Africa, epidemiology, lyssavirus, rabies-related lyssavirus

1. Introduction

Although rabies is appreciated as an animal disease, it is one of the most significant viral zoonoses of all time and continues to affect tens of thousands of people worldwide. The burden is particularly high in the developing world – most notably the majority of countries in Africa and Asia, where dog rabies control is generally nonexistent or completely inadequate (Knobel et al., 2005, Dodet, 2009). Therefore, given the longstanding availability of effective prevention strategies in humans and control strategies in vectors and reservoirs of the disease, the expansion of the rabies problem over large areas of the world emphasizes the seriously neglected state of this disease.

In the vast majority of cases, rabies, is caused by infection with the rabies virus (RABV) belonging to the genus *Lyssavirus*, family *Rhabdoviridae*. However, to date an additional ten species of lyssaviruses, also known as rabies-related viruses, have been formally classified. These are Lagos bat virus (LBV), Mokola virus (MOKV), Duvenhage virus (DUVV), European bat

lyssavirus type 1 (EBLV-1), European bat lyssavirus type 2 (EBLV-2), Australian bat lyssavirus (ABLV) and the four most recently recognized species; Aravan, Khujand, Irkut and West Caucasian bat virus (WCBV) (Arai et al., 2003; Botvinkin et al., 2003; Kuzmin et al., 2003, 2005 and 2008b; ICTV, 2009). In addition, Shimoni virus, a recently described putative lyssavirus species has been isolated from an insectivorous bat (*Hipposideros commersoni*) found in a cave in Kenya (Kuzmin et al., 2010). Cases of infection with RABV, LBV, MOKV and DUVV have been reported from the African continent, with the latter three occurring exclusively in Africa (Nel and Rupprecht, 2007). Improved surveillance across the continent is required to fully appreciate the epidemiology of these viruses. In addition, serological evidence of predominantly ABLV infection in bats from several Asian countries and WCBV in bats collected in Kenya (with only known isolate from Asia) predicts a more widespread distribution of these viruses than may have been anticipated (Arguin et al., 2002; Reynes et al., 2004; Lumlerthdacha et al., 2005; Kuzmin et al.,2008c).

In the Republic of South Africa (RSA) two major variants of RABV are distinguished and these circulate in *Canidae* and *Herpestidae* species, respectively (King et al., 1993; Von Teichman et al., 1995; Nel et al., 2005). The canid RABV variant occurs widespread in the RSA and is mainly associated with the domestic dog (*Canis familiaris*) in the KwaZulu Natal, Eastern Cape, Free State, Mpumalanga and Limpopo Provinces (Coetzee and Nel, 2007, Ngoepe et al., 2009), black-backed jackal (*Canis mesomelas*) in the North West (Zulu et al., 2009), Mpumalanga and Limpopo Provinces and bat-eared fox (*Otocyon megalotis*) in the Western and Northern Cape Provinces (Sabeta et al., 2007a). This canid RABV variant is closely related to European and cosmopolitan viruses (Von Teichman et al., 1995) and the introduction and spread of this canid RABV in sub-Saharan Africa correlates with colonial period activities of the late 19th and early 20th century (Nel and Rupprecht, 2007). Most significantly for the RSA, an epidemiological cycle in dogs was established in the KwaZulu Natal Province, firstly in the 1950s and once again in the early 1980s (Swanepoel, 2004). In addition, canid rabies has recently re-emerged in several provinces of the RSA with sizable

outbreaks of the disease reported in the Free State Province since 2000, Limpopo Province after 2005 and Mpumalanga Province since 2008 (Cohen et al., 2007; Ngoepe et al., 2009). On the other hand, the mongoose RABV variant circulates in several herpestid species scattered over the central plateau of the country. Reports of exposures to rabid mongooses dates back to the 1800s, and together with phylogenetic data and the genetic diversity of this group of viruses, it has been proposed that this mongoose RABV variant may have been introduced to southern Africa earlier than the cosmopolitan RABV variant (Swanepoel, 2004; Nel et al., 2005). Molecular clock analysis suggests that a common ancestor for the two RABV variants dates back about 200 years on average depending on the gene analysed (van Zyl et al., 2010).

The most common member of the three African rabies-related lyssaviruses, LBV, has been sporadically isolated for several species of fruit bat, one insectivorous bat, domestic cats, domestic dogs and a water mongoose (*Atilax paludinosus*) from various African countries, but predominantly from the RSA (Markotter et al., 2006a and b; Markotter et al., 2008; Kuzmin et al., 2008a). Although indications are that some LBV isolates or variants are highly pathogenic in mouse models compared to invasive RABV strains, no confirmed human cases have been linked to infection with the virus as of yet (Markotter et al., 2009). Among the lyssaviruses the epidemiology of MOKV is particularly obscure and the virus has been infrequently isolated from several animal species, including domestic dogs, cats and shrews in the past 30 years (Sabeta et al., 2007b). On various occasions, cases of fatal MOKV infections in dogs and cats occurred in animals with rabies vaccination histories (Nel et al., 2000, Sabeta et al., 2009) however MOKV has not been associated with definitive human rabies cases (Familusi and Moore, 1972, Familusi et al., 1972). Importantly MOKV has been associated with rabies in several animals with rabies vaccination history and may pose a more significant public health threat than anticipated (Sabeta et al., 2009). The most elusive African lyssavirus, the DUVV, has only been reported five times since its original description in the late 1970s. Strikingly three of the five cases were associated with fatal human infections, with two of these cases reported in the past three years (Paweska et al., 2006, van Thiel et al., 2009). The most recent human

rabies case associated with DUVV infection outside of southern Africa corroborates a broader distribution of the virus in sub-Saharan Africa (van Thiel et al., 2009).

Human rabies cases have been anecdotally reported in the RSA since the 1800s, but the first cases were only confirmed in 1928 following exposures to a rabid mongoose (Swanepoel, 2004). Thereafter human rabies cases have been confirmed intermittently but have increased in numbers since the 1980s after the establishment of rabies in domestic dogs in the KwaZulu Natal Province and lately in a number of other provinces as indicated above. This paper reports on the epidemiology of human rabies in the RSA, as well as molecular characterization of the viruses isolated from cases confirmed over a 25 year period (1983 to 2007).

2. Materials and methods

2.1 Case definition and data source, extraction and analysis

The study included all laboratory confirmed human rabies cases for the RSA for the period 1983-2007. The National Institute for Communicable Diseases of the National Health Laboratory Service is the sole centre for human rabies testing in the RSA and cases were confirmed by performing the direct fluorescent antibody test performed on brain impressions (Dean et al., 1996) and/or reverse transcription PCR (Heaton et al., 1997) and/or virus isolation in suckling mice (Koprowski, 1994). Case history data were extracted from the case notes for each case and analysed with EpiInfo 3.5.1 (http:www.cdc.gov/epiinfo/). Ethics clearance (reference number M090120) for the use of the case history data and clinical specimens (next section) was obtained from the University of the Witwatersrand Human Ethics Committee.

2.2 Virus isolates

A panel of 127 virus isolates was analysed in this study. The virus isolates were available as lyophilized material or suckling mouse brain homogenates.

2.3 PCR, sequencing and phylogenetic analysis

Nucleic acid was extracted using the guanidium-isothiocyanate method with Trizol® reagent (Invitrogen, USA) according to the manufacturer's suggestions. RT-PCR targeting the G-L intergenic region of the RABV genome (Von Teichman et al., 1995) or an additional pan-lyssavirus RT-PCR (Markotter et al., 2006) was used as previously described. The RT-PCRs were prepared with the Titan® One Tube RT PCR system (Roche, Germany). Amplicons were subsequently purified and sequenced using the V3.1 BigDye® Terminator System (Applied Biosystems, USA).

Phylogenetic tree re-construction was carried out incorporating previously published representative RABV sequences (Table 1) and the sequences generated from the isolates available for this study (Table 1) using Mega 4.0 software (Tamura et al., 2007). The Kimura 2-parameter method (Kimura, 1980) was used to determine genetic distance and subsequently to calculate neighbour-joining trees. The branching order of the trees was established by 1000 bootstrap replicates.

3. Results

3.1 Epidemiological trends of human rabies in the RSA

3.1.1 Case distribution

A total of 353 laboratory confirmed human rabies cases from the RSA were analysed for this study. The majority (n=279) of the cases were reported from the KwaZulu Natal Province, but cases were also reported from the Eastern Cape (n=29), Limpopo (n=23), Free State (n=8), Mpumalanga and North West (n=5 each), Northern Cape (n=3) and Gauteng (n=1) provinces (Figure 1a). The number of cases from KwaZulu Natal dropped markedly after 1995 with less than 10 cases confirmed annually. In contrast, the number of cases reported from the Eastern Cape Province has increased from single cases reported intermittently to several cases confirmed each year since 2005. The spike in number of cases in the Limpopo Province in 2006 was attributed to

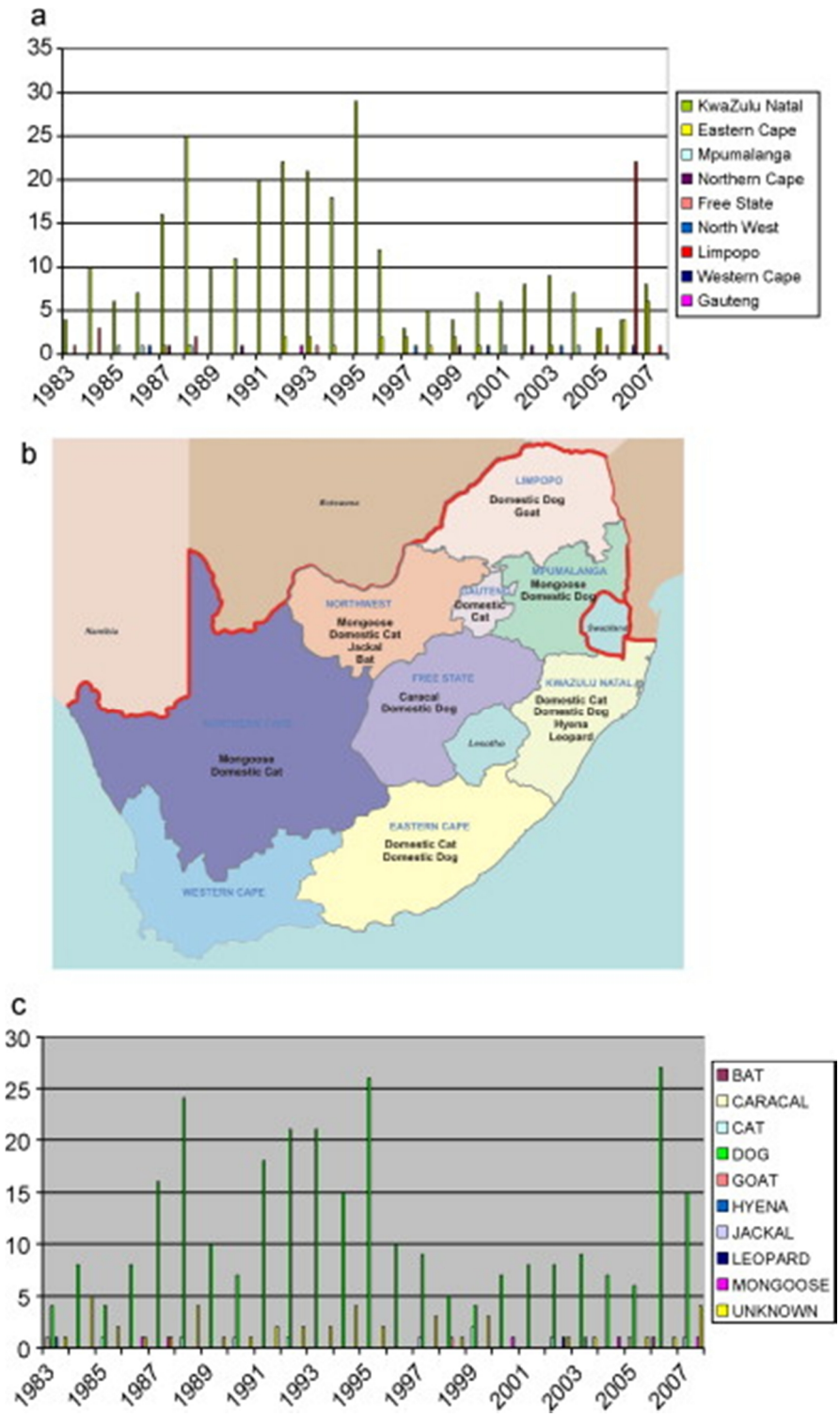

Fig. 1. Human rabies in South Africa, 1983–2007. (a) Provincial breakdown of confirmed human rabies cases in South Africa for the period 1983–2007. (b) A map of South Africa indicates the nine provinces of the country and the vectors that have been associated with human rabies cases in each province. (c) Breakdown of the vectors associated with exposures in confirmed human cases.

Table 1: Previously published sequences representing the phylogenetic spectrum of RABV in Southern Africa were used in this study.

No	Genbank accession number	Geographic location	Biotype/Phylogenetic grouping	Reference
1	EF686085	Limpopo	Canid	Cohen et al. 2007
2	EF686098	Limpopo	Canid	Cohen et al. 2007
3	EF686136	Limpopo	Canid	Cohen et al. 2007
4	EF686143	Limpopo	Canid	Cohen et al. 2007
5	EF686128	Limpopo	Canid	Cohen et al. 2007
6	EF686086	Mpumalanga	Canid	Cohen et al. 2007
7	EF686125	Mpumalanga	Canid	Cohen et al. 2007
8	EF686051	North West	Canid	Cohen et al. 2007
9	AF177107	North West	Canid	Cohen et al. 2007
10	DQ431351	Northern Cape	Canid	Cohen et al. 2007
11	DQ431364	Western Cape	Canid	Cohen et al. 2007
12	DQ841546	KZN	Canid	Coetzee et al. 2007
13	DQ841548	KZN	Canid	Coetzee et al. 2007
14	DQ841549	KZN	Canid	Coetzee et al. 2007
15	DQ841423	KZN	Canid	Coetzee et al. 2007
16	DQ841547	Eastern Cape	Canid	Coetzee et al. 2007
17	DQ841488	KZN	Canid	Coetzee and Nel, 2007
18	DQ841516	KZN	Canid	Coetzee and Nel, 2007
19	DQ841446	KZN	Canid	Coetzee and Nel, 2007
20	DQ841426	KZN	Canid	Coetzee and Nel, 2007
21	DQ841431	KZN	Canid	Coetzee and Nel, 2007
22	DQ841542	KZN	Canid	Coetzee and Nel, 2007
23	DQ841500	KZN	Canid	Coetzee and Nel, 2007
24	DQ841408	Eastern Cape	Canid	Coetzee and Nel, 2007
25	DQ841404	Eastern Cape	Canid	Coetzee and Nel, 2007
26	DQ841512	KZN	Canid	Coetzee and Nel, 2007
27	DQ841481	KZN	Canid	Coetzee and Nel, 2007
28	AF304188	Zimbabwe	Mongoose	Nel et al. 2005
29	AF079907	South Africa	Mongoose	Nel et al. 2005
30	AY353993	South Africa	Mongoose	Nel et al. 2005
31	AF079932	South Africa	Mongoose	Nel et al. 2005
32	AF079914	South Africa	Mongoose	Nel et al. 2005
33	EU163361	Free State	Canid	Ngoepe et al. 2009
34	EU163310	Free State	Canid	Ngoepe et al. 2009
35	EU163323	Free State	Canid	Ngoepe et al. 2009
36	EU163339	Free State	Canid	Ngoepe et al. 2009
37	EU163341	Free State	Canid	Ngoepe et al. 2009
38	EU163328	Free State	Canid	Ngoepe et al. 2009

the re-emergence of rabies in dogs in this region. No human cases were confirmed from the Western Cape during the study period. Nearly two thirds of the cases were in male patients aged between 1 to 85 years (Table 3). More than 70 % of the cases were reported in children and young adults (Table 3).

Table 3: Summary of demographics of confirmed human rabies cases in South Africa, 1983-2007

Feature	Number (Percentage of total)
Sex	
Male	228 (64.6%)
Female	110 (31.2%)
Unreported	15 (4.2%)
Age	
Children (<10 years)	172 (48.8%)
Young adults (11-20 years)	77 (21.8%)
Adults (>20 years)	89 (25.2%)
Unreported	15 (4.2%)

3.1.2 Sources of exposure

The domestic dog was implicated as the major source of exposure (84 %) linked to the cases analysed with only single cases of reported wildlife involvement (Figure 1b). Despite the prevalence of mongoose rabies in the RSA only 4 cases reported an exposure to a mongoose during the study period. A single case was attributed to a bat exposure in 2006 which led to an infection with DUVV as previously reported (Paweska et al., 2006).

3.1.3 Use of rabies post exposure prophylaxis

Information regarding the prophylaxis that patients received was not recorded consistently. The most complete subset of data was for 2007 and this was

analyzed for trends in the application of post exposure prophylaxis and to elucidate the causality of the case management failure. In this period a total of 46 % of the 15 cases (n=7) apparently did not seek any medical consultation after the animal exposure. The second most prevalent situation (40% or n=6) was when patients presented to health care facilities for medical attention but only received primary wound care and/or tetanus toxoid after the animal exposure. In one case the patient did not complete the prophylaxis schedule after receiving only one dose of rabies vaccine. In the remaining case no details of prophylaxis were recorded.

3.1.4 Clinical features and hospitalization

Clinical features of 148 cases were recorded and available for analysis (Table 2). It is noteworthy that the data were not collected and recorded on standard questionnaires, but extracted from patient case notes. Features that could be described as behavioural changes in patients accounted for the most frequently noted symptoms. Altered mental state - including confusion and hallucinations, were recorded in 38.5 % of patients and restlessness in a further 20.9%. Hydrophobia (and difficulty in swallowing which was interpreted as a sign of hydrophobia) was noted in 52% of the cases. Hypersalivation was also recorded in about a third of the cases. Only 20 cases had records for incubation period. The range of reported incubation period was between 1-131 days with a mean of 53.8 days ± 3.75 standard deviations (SD). Duration of hospitalization was recorded in 184 of the cases and the range of hospital stay was 0-31 days with a short median of 1 day ± 3.8 SD.

3.2 Molecular diversity of lyssavirus causing human rabies in the RSA

Molecular analysis of lyssavirus isolates obtained from human rabies cases supported the molecular analysis of RABV from veterinary sources in the RSA (Tables 2 and 4, Figure 2) (Coetzee and Nel, 2007; Cohen et al., 2007, Ngoepe et al., 2009, Sabeta et al., 2007; Zulu et al., 2009). In these previous studies, related to the phylogeny of Southern African rabies virus isolates, the G-L intergenic region has been most extensively used and therefore chosen

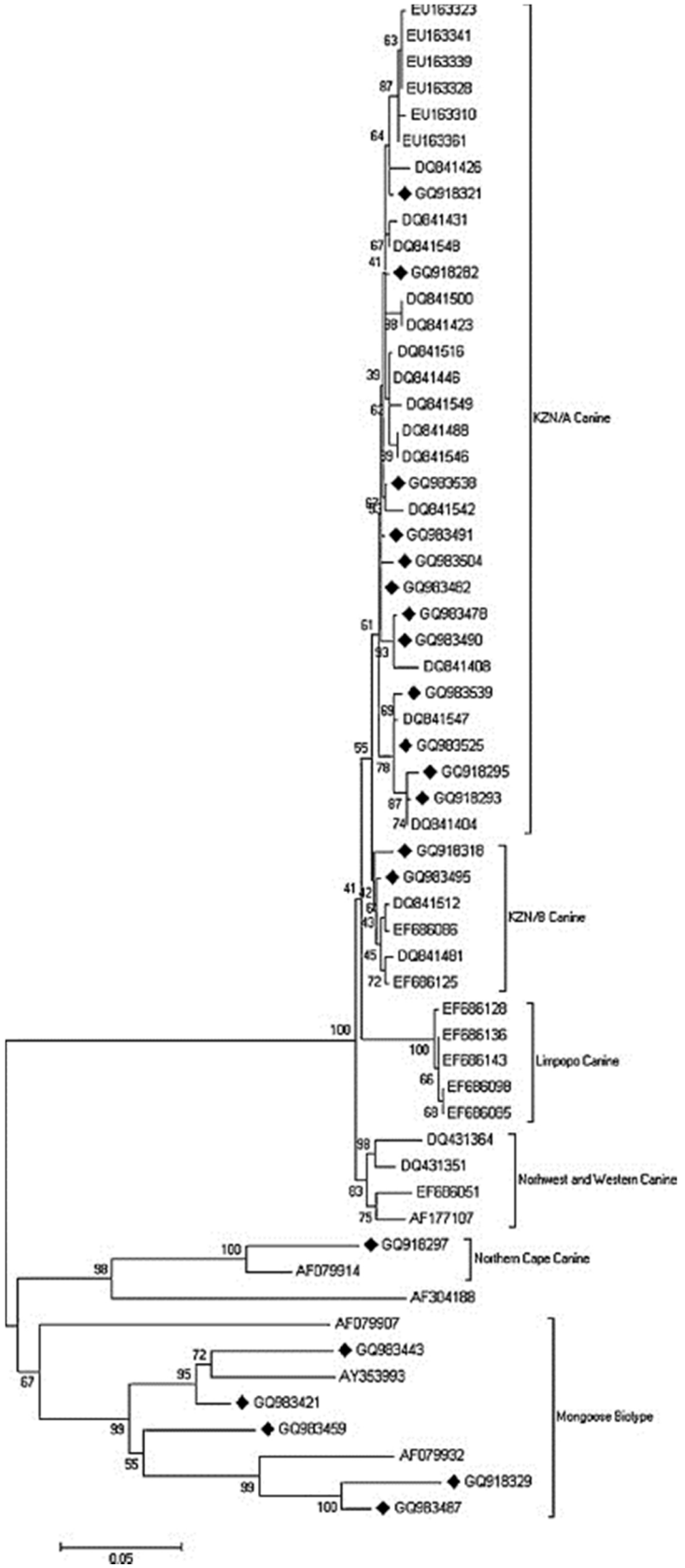

Fig. 2. An unrooted neighbour-joining tree of the G-L intergenic region of rabies virus isolates of human rabies cases from South Africa. To simplify the tree, the largest subset of sequences obtained from cases from the KwaZulu Natal province clustered that clustered with the KZN/A group was obviated here. The isolates investigated in this study are indicated with diamonds.

Table 2. Summary of isolates/samples analyzed in this study.

Number	Laboratory reference number	Year and geographic location	Animal involved in exposure	Genbank accession number
1	133.83	1983, Mkuze/KZN	Dog	GQ918301
2	147.83	1983, Unknown/Unknown	Unknown	GQ918313
3	181.83	1983, Stanger/KZN	Dog	GQ983393
4	190.84	1984, Inanda/KZN	Dog	GQ983397
5	366.84	1984, Ladybrand/Free State	Unknown	GQ983482
6	368.84	1984, Unknown/Unknown	Dog	GQ983483
7	393.84	1984, Ladybrand/Free State	Dog	GQ983491
8	406.84	1984, Umphumulo/KZN	Unknown	GQ983493
9	486.84	1984, Unknown/Free State	Unknown	GQ983510
10	360.85	1985, Bomvaneni/KZN	Dog	GQ983480
11	11.86	1986, Botha's Hill/KZN	Dog	GQ918287
12	151.86	1986, Lower Creek/Mpumalanga	Dog	GQ918318
13	158.86	1986, Camperdown/KZN	Dog	GQ918322
14	267.86	1986, Potchefstroom/North West	Mongoose	GQ983443
15	326.86	1986, Matatiele/KZN	Dog	GQ983472
16	383.86	1986, Matatiele/KZN	Dog	GQ983489
17	759.86	1986, Amanzintoti/KZN	Unknown	GQ983534
18	265.87	1987, Marionhill/KZN	Dog	GQ983440
19	378.87	1987, Witputs/Northern Cape	Mongoose	GQ983487
20	452.87	1987, Umlazi/KZN	Dog	GQ983503
21	468.87	1987, Inanda/KZN	Dog	GQ983505
22	48.87	1987, Etsheni/KZN	Dog	GQ983508
23	586.87	1987, Umzinto/KZN	Dog	GQ983519
24	74.87	1987, Umbumbulu/KZN	Dog	GQ983532
25	14.88	1988, Inanda/KZN	Dog	GQ918309
26	172.88	1988, Ndwedwe/KZN	Unknown	GQ918331

Number	Laboratory reference number	Year and geographic location	Animal involved in exposure	Genbank accession number
27	239.88	1988, Ashwood/KZN	Dog	GQ983425
28	276.88	1988, Izingolweni/KZN	Dog	GQ983450
29	292.88	1988, Greytown/KZN	Dog	GQ983458
30	341.88	1988, Umlazi/KZN	Dog	GQ983477
31	353.88	1988, Transkei/EC	Dog	GQ983478
32	373.88	1988, Umbumbulu/KZN	Dog	GQ983486
33	385.88	1988, Transkei/EC	Dog	GQ983490
34	411.88	1988, Shongwe/Mpumalanga	Dog	GQ983495
35	424.88	1988, Maphumulo/KZN	Dog	GQ983499
36	455.88	1988, Fouriesberg/KZN	Dog	GQ983504
37	476.88	1988, Chatsworth/KZN	Dog	GQ983507
38	540.88	1988, Izingolweni/KZN	Dog	GQ983515
39	585.88	1988, Botha's Hill	Unknown	GQ983518
40	84.88	1988, Ficksburg/Free State	Dog	GQ983538
41	195.89	1989, Ezingolweni/KZN	Dog	GQ983400
42	3.89	1989, Shongweni/KZN	Unknown	GQ983464
43	30.89	1989, Eshowe/KZN	Dog	GQ983465
44	438.89	1989, Empangeni/KZN	Dog	GQ983501
45	242.90	1990, Umbumbulu/KZN	Dog	GQ983427
46	250.90	1990, Richmond/KZN	Dog	GQ983430
47	292.90	1990, Kuruman/Northern Cape	Cat	GQ983459
48	326.90	1990, Stanger/KZN	Dog	GQ983473
49	42.90	1990, Ntuzuma/KZN	Dog	GQ983496
50	104.91	1991, Pietermaritzburg/KZN	Dog	GQ918283
51	168.91	1991, Ematimatolo/KZN	Dog	GQ918327
52	176.91	1991, Umzinto/KZN	Dog	GQ983385
53	52.91	1991, Inkanyezi/KZN	Dog	GQ983512
54	77.91	1991, Ndwedwe/KZN	Unknown	GQ983535
55	88.91	1991, Inkayezi/KZN	Dog	GQ983540
56	93.91	1991, Camperdown/KZN	Dog	GQ983542
57	2.92	1992, Camperdown/KZN	Unknown	GQ918281
58	114.92	1992, Clermont/KZN	Dog	GQ918289

Number	Laboratory reference number	Year and geographic location	Animal involved in exposure	Genbank accession number
59	126.92	1992, Umlazi/KZN	Dog	GQ918296
60	133.92	1992, Hlabisa/KZN	Dog	GQ918302
61	144.92	1992, Inkanyezi/KZN	Dog	GQ918311
62	171.92	1992, Eshowe/KZN	Dog	GQ918330
63	224.92	1992, Ntuzuma/KZN	Dog	GQ983413
64	232.92	1992, Vereeniging/Gauteng	Cat	GQ983421
65	261.92	1992, Ingwavuma/KZN	Dog	GQ983437
66	262.92	1992, Maphumulo/KZN	Dog	GQ983438
67	291.92	1992, Ubombo/KZN	Dog	GQ983457
68	87.92	1992, Ngcora/Transkei	Dog	GQ983539
69	103.93	1993, Hoopstad/Free State	Dog	GQ918282
70	106.93	1993, Maphumulo/KZN	Dog	GQ918284
71	115.93	1993, Msinga/KZN	Dog	GQ918290
72	13.93	1993, Inanda/KZN	Dog	GQ918298
73	134.93	1993, Ozwathini/KZN	Dog	GQ918303
74	164.93	1993, Vulamehlo/KZN	Dog	GQ918325
75	165.93	1993, Msinga/KZN	Dog	GQ918326
76	225.93	1993, Pinetown/KZN	Dog	GQ983415
77	230.93	1993, Thabankulu/KZN	Dog	GQ983420
78	247.93	1993, Ixopo/KZN	Dog	GQ983428
79	334.93	1993, Nkandla/KZN	Dog	GQ983474
80	109.94	1994, Vulindlela/KZN	Dog	GQ918286
81	15.94	1994, Pietermaritzburg/KZN	Dog	GQ918317
82	25.94	1994, Empumalanga/KZN	Dog	GQ983429
83	319.94	1994, Umzinto/KZN	Unknown	GQ983470
84	64.94	1994, Ubumbulu/KZN	Dog	GQ983522
85	73.94	1994, Lower Umfolozi/KZN	Dog	GQ983531
86	130.95	1995, Inanda/KZN	Unknown	GQ918299
87	14.95	1995, Umzinto/KZN	Dog	GQ918310
88	155.95	1995, Ndwedwe/KZN	Dog	GQ918320
89	169.95	1995, Port	Dog	GQ918328

Number	Laboratory reference number	Year and geographic location	Animal involved in exposure	Genbank accession number
		Shepstone/KZN		
90	18.95	1995, Umzinto/KZN	Dog	GQ983390
91	20.95	1995, Weenen/KZN	Dog	GQ983403
92	27.95	1995, Umlazi/KZN	Dog	GQ983442
93	311.95	1995, Maphumulo/KZN	Dog	GQ983468
94	178.96	1996, Camperdown/KZN	Dog	GQ983387
95	397.96	1996, Stanger/KZN	Dog	GQ983492
96	75.96	1996, Enseleni/KZN	Dog	GQ983533
97	9.96	1996, Nongoma/KZN	Dog	GQ983541
98	232.97	1997, Inanda/KZN	Dog	GQ983422
99	137.98	1998, Kranskop/KZN	Dog	GQ918307
100	216.98	1998, Eshowe/KZN	Dog	GQ983410
101	129.99	1999, Kuruman/Northern Cape	Cat	GQ918297
102	135.99	1999, Richmond/KZN	Unknown	GQ918306
103	156.99	1999, Bizana/EC	Dog	GQ918321
104	71.99	1999, Eshowe/KZN	Cat	GQ983527
105	257.00	2000, Paulpietersburg/KZN	Dog	GQ983436
106	198.01	2001, Enseleni/KZN	Dog	GQ983401
107	255.01	2001, Empangeni/KZN	Dog	GQ983434
108	284.01	2001, Kwambonambi/KZN	Dog	GQ983452
109	7.01	2001, Tsomo/EC	Dog	GQ983525
110	199.02	2002, Ndwedwe/KZN	Dog	GQ983402
111	294.02	2002, Eshowe/KZN	Dog	GQ983461
112	15.03	2003, Lower Umfolozi/KZN	Dog	GQ918316
113	272.03	2003, Tugela Ferry/KZN	Dog	GQ983447
114	288.03	2003, Manguzi/KZN	Dog	GQ983454
115	63.03	2003, Eshowe/KZN	Dog	GQ983520
116	161.04	2004, Mthunzini/KZN	Dog	GQ918323
117	126.05	2005, Ngqamakwe/EC	Dog	GQ918295
118	17.05	2005, Jagersfontein/Free State	Caracal	GQ918329
119	183.05	2005, Umzimkulu/KZN	Dog	GQ983394

Number	Laboratory reference number	Year and geographic location	Animal involved in exposure	Genbank accession number
120	218.05	2005, Kwabangibizo/KZN	Unknown	GQ983414
121	267.06	2006, Hibiscus/KZN	Dog	GQ983441
122	70.06	2006. Scottburgh/KZN	Dog	GQ983526
123	125.07	2007, Ngcobo/EC	Dog	GQ918293
124	228.07	2007, Mthatha/Eastern Cape	Dog	GQ983417
125	268.07	2007, Umhlatuze/KZN	Dog	GQ983444
126	317.07	2007, Umgungundlovu/KZN	Dog	GQ983469
127	46.07	2007, Empangeni/KZN	Dog	GQ983502

Table 4. Summary of clinical features recorded for confirmed human rabies cases in South Africa, 1983–2007.

Clinical feature	Frequency noted (percentage of total)
Behavioural	
Aerophagia	9 (6.1%)
Aerophobia	6 (4.1%)
Aggression	13 (8.8%)
Altered mental state (confusion/hallucinations/disorientation/psychosis/delirium)	57 (38.5%)
Barking	1 (0.7%)
Biting	1 (0.73%)
Hydrophobia/difficulty swallowing	77 (52%)
Insomnia	2 (1.4%)
Restlessness/anxiety	31 (20.9%)
Gastrointestinal	
Abdominal pain	3 (2.0%)
Diarrhea	3 (2.0%)
Nausea/vomiting	25 (16.9%)
Respiratory	
Respiratory distress	3 (2.0%)
Other	
Coma	1 (0.7%)
Convulsions	8 (5.4%)
Decreased reflexes	1 (0.7%)
Dizziness	1 (0.7%)
Drowsiness	1 (0.7%)
Dry mouth	1 (0.7%)
Excessive sweating	2 (1.4%)
Flaccid paralysis	6 (4.1%)
Headache	8 (5.4%)
Hypersalivation	45 (30.4%)
Neck stiffness	4 (2.7%)
Pyrexia	8 (5.4%)
Slurred speech	2 (1.4%)
Weakness	8 (5.4%)

as the target in this study. The majority of virus sequences in our collection corresponded with the canine variant RABV cluster previously designated as KZN/A and EC/A (Coetzee and Nel, 2007). This is the largest and least differentiated group and contains sequences from specimens that originated from the KwaZulu Natal, the Free State and Eastern Cape Provinces. Notably the RABV associated with human cases in the Free State Province did not appear to have originated from the same epidemiological cycle as the representative canine cases from the same area. Canine RABV derived sequences grouped closely together within the KZN/A cluster, but RABV sequences derived from human cases grouped separately from these sequences and were dispersed throughout the larger KZN/A cluster. In addition, a second smaller cluster was formed that grouped closely with the KZN/B group of canine RABV, but also contained sequences derived from human rabies cases in KwaZulu Natal and Mpumalanga provinces. Phylogenetically the canine RABV from cases in the Northern and Western Cape provinces were also distinct from those associated with other regions of the RSA. The Limpopo human cases previously published (Cohen et al. 2007) were clearly linked with the canine cycle of the corresponding area.

Of the four cases that reported mongoose exposures in the study period, only two isolates (267/86 and 378/87) were available for molecular typing and both isolates were found to be of the mongoose RABV variant. However, the study also identified three additional cases that were caused by the mongoose RABV variant. These included two reported domestic cat exposures, one reported from the Gauteng Province (232/92) and the other from the Northern Cape Province (292/90). The third case was associated with a caracal (*Caracal caracal*) exposure from the Free State (17/05).

Apart from the single DUVV case (Paweska et al., 2006); our results did not reveal any additional cases of LBV, MOKV or DUVV virus infection in the study period.

4. Discussion

Here we have described that the majority of human rabies cases in the RSA have been due to dog exposures and that the failure to apply post exposure prophylaxis primarily stemmed from a lack of awareness concerning the need and importance of such treatment, not only among the public, but also among health care providers. This is a scenario that is likely to prevail in any other country where canine rabies is endemic. Secondly, rabies primarily affects children, with almost 70 % of the cases recorded for individuals below the age of 20 and with more than two thirds of the cases in males. The latter observations are in agreement with other studies of human rabies in not only developing countries (e.g. Pfukenyi et al., 2007), but also the developed world (e.g. Noah et al., 1998).

The documented clinical features of human rabies cases encountered over 25 years in the RSA were found to show little deviation from those features described for a recent outbreak (2005-2006) in one of the provinces, the Limpopo Province, with the previous reports of human rabies cases dates back to before 1983 (Cohen et al., 2007). Although an altered mental state and behavioural changes were some of the most frequently noted symptoms, the range of symptoms highlighted the difficulty of clinical recognition and underpins the need to consider rabies as a differential diagnosis in all viral encephalitides in rabies endemic areas. This point was well illustrated in the case of the Limpopo Province outbreak in 2005-2006, where rabies was first diagnosed several months after the actual onset of the epidemic (Cohen et al., 2007).

The duration of hospitalization of human rabies cases was found to average only one day with patients presenting to health care facilities just prior to their demise. This is in contrast with what is reported in the United States where most of the cases receive up to two weeks of intensive supportive treatment (Noah et al., 1998). This late presentation to health care facilities certainly negates the application of protocols for the experimental treatment of rabies (Nig et al., 2009, The Wisconsin College of Medicine, 2009). Last minute

presentation to healthcare facilities may not be unexpected, given particular infrastructural and socioeconomic considerations, but the late presentation of rabies cases may in some cases also be attributed to the role of indigenous medicine. This was found during the Limpopo-outbreak (Cohen et al., 2007) and although not specifically documented in the cases studied here, the influence of traditional healers is a well known phenomenon in southern Africa (Coovadia et al., 2009).

The RABV isolates from human cases in the RSA were phylogenetically matched with those viruses characterized for animal rabies cycles in the country. Indeed, the majority of virus sequences placed these cases within the homogenous, low diversity cluster previously dubbed KZN/A, correspondent with their locations of origin. All of the human rabies cases identified from the Free State Province were reported prior to 2000. These cases did not cluster with the canine RABV isolates available from the same province, since all of these are from more recent outbreaks or cycles (canine rabies was introduced into Free State in 1998 from the Kingdom of Lesotho) (Ngoepe et al., 2009). Alarmingly no human cases have been reported from this province since 2000 despite the rise in dog rabies cases which is most likely an indicative of a lack of awareness in this area.

The contribution of rabies-related lyssaviruses to the public health burden of rabies has long been the topic of speculation. Although these viruses have not been associated with a great number of human (and animal) cases the potential threat has been anticipated (Nel and Rupprecht, 2007). The majority of rabies-related lyssaviruses have been reported from African and/or Eurasian countries where surveillance is generally low. Traditional diagnostic methods (including the fluorescent antibody test, virus isolation and conventional nucleic acid detection based assays) do not allow for differentiation between the lyssavirus types without further manipulation. Despite the proven co-circulation of several lyssavirus species in the RSA, this retrospective molecular study did not identify any additional cases of human rabies attributed to infection with DUVV, or any cases associated with MOKV or LBV. To date DUVV has been associated with three fatal human

rabies cases. Although the bat reservoir of DUVV remains to be proven indications are that insectivorous bats are involved (Nel and Markotter, 2007). All three human cases were associated with abnormal behaviour of these bats involved including the animals entering human dwellings and flying into the victims (Paweska et al., 2006, van Thiel et al., 2009). Similarly EBLV-1 and 2 and ABLV have only been associated with four and two human cases to date respectively (Warrell and Warrell, 2004). It is noteworthy that half of these cases involved included people with regular close contact with bats including bat handlers. Interestingly, several studies involving bats from different Asian countries have shown evidence of virus neutralizing antibodies against rabies-related viruses (most commonly ABLV) in up to 19 % of the samples tested (Arguin et al., 2002; Reynes et al., 2004; Lumlertdacha et al., 2005). Although the majority of human rabies cases in the world are reported from Asian countries no evidence has been reported for infection with rabies related viruses in these cases.

The lack of rabies cases associated with infection of these rabies-related lyssaviruses are likely due to the lack of usual direct contact (for example bites, scratches) between the bat species involved and the human population in these areas despite these bats often roosting in densely populated areas. This situation may be different in countries where there is more human interaction with bats (for example where bats are hunted as a food source or are more closely associated with rural human habitats) and will also depend on the bat species involved and its distribution.

While dogs remain the major vector of human rabies in the RSA, only a few cases reported exposures to domestic cats, as cats are incidental victims of rabies and due to behavioural characteristics do not support independent rabies cycles (Pfukenyi et al., 2007; Lackay et al., 2008). In addition, very little wildlife involvement has been reported despite the established cycles of rabies in various wildlife species in the region. This observation was also made in a similar study of human rabies in Zimbabwe where similar urban and sylvatic cycles of rabies exist (Pfukenyi et al., 2007). Over the entire study period, only four cases reported exposure to rabid mongooses, with the virus

genome sequences conforming to the mongoose variant, despite the known prevalence of rabies in these animals. Prior to the establishment of canine rabies in the KwaZulu Natal Province and later in various other provinces, up to 25 % of the confirmed human rabies cases in the RSA were attributed to mongoose exposures (Swanepoel, 2004). Therefore, the dominance of dogs as rabies reservoir and vector, despite the co-circulation of RABV and rabies-related viruses in several wildlife species, has been demonstrated. Other sources of data, including dog bite registers and reliable documentation systems for post exposure prophylaxis usage is required in order to provide a comprehensive description of the burden of rabies in the RSA. However, it is notable that human rabies has been on the decrease in KwaZulu Natal, a province where dog rabies control programmes had been implemented for several years. While this program is being expanded towards elimination of dog rabies altogether, the KwaZulu Natal Province is being overtaken by several other provinces (the Eastern Cape and Mpumalanga) as the main foci of epidemic dog rabies in the face of less ideal efforts.

Acknowledgements

The authors would like to thank Miss Shantal Pillay of the Editorial and Photographic Unit of the NHLS for producing the figures used in this manuscript and the technical team of the Special Pathogens Unit, NICD-NHLS for assisting in the nucleic acid extraction of samples. This study was funded by a grant from the Poliomyelitis Research Fund (grant number 07/11) and ASP was supported by a National Research Foundation bursary.

References

Arai, Y.T., Kuzmin, I.V., Kameoka, Y., Botvinkin, A.D., 2003. New lyssavirus genotype from the lesser mouse-eared bat (*Myotis blythi*), Kyrgyzstan. Emerg. Infect. Dis. 9(3), 333-337

Arguin, P.M., Murray-Lillibridge, K., Miranda, M.E.G., Smith, J.S., Calaor, A.B., Rupprecht, C.E., 2002. Serological evidence of lyssavirus infections among bats the Philippines. Emerg. Infect. Dis. 8(3), 258-262

Botvinkin, A.D., Poleschuk, E.M., Kuzmin, I.V., Gazaryan, S.V., Yager, P., Rupprecht, C.E., 2003. Novel lyssaviruses isolated from bats in Russia. Emerg. Infect. Dis. 9 (12), 1623-1625

Coetzee, P., Nel, L.H., 2007. Emerging epidemic dog rabies in coastal South Africa: a Molecular epidemiological analysis. Virus Res. 126, 186-195

Cohen, C., Sartorius, B., Sabeta, C., Zulu, G., Paweska, J., Mogoswane, M., Sutton, C., Nel, L.H., Swanepoel, R., Leman, P.A., Grobbelaar, A.A., Dyason, E., Blumberg, L., 2007. Epidemiology and viral molecular characterization of reemerging rabies, South Africa. Emerg. Infect. Dis. 13(12), 1879-1886

Coovadia, H., Jewkes, R., Barron, P., Sanders, D., McIntyre, D., 2009. The health and health system of South Africa: historical roots of current public health challenges. The Lancet, 374 (9692), 817-834

Dean, D.J., Abelseth, M.K., Atanasiu, P., 1996. The fluorescent antibody test. In: Meslin, F.-X., Kaplan, M.M., Koprowski, H (Eds). Laboratory techniques in rabies, 4th Edn. World Health Organization, Geneva, pp. 88-89

Dodet, B., Africa Rabies Bureau (AfroREB)., 2009. The fight against rabies in Africa: from recognition to action. Vaccine, 27 (37), 5027-5032

Heaton P.R., Johnstone P., McElhinney L.M., Cowley R., O'Sullivan E., Whitby J.E., 1997. Hemi-nested PCR assay for detection of six genotypes of rabies and rabies-related viruses. J. Clin. Microbiol. 35(11), 2762-2766.

ICTV Official Taxonomy Updates since the 8th report. Vertebrate.

http://talk.ictvonline.org/media/p/1208.aspx [accessed 2009/1/24]

Familusi, J.B., Moore, D.L., 1972. Isolation of rabies-related virus from the cerebrospinal fluid of a child with aseptic meningitis. African J. Med. Sci. 3, 93-96

Familusi, J.B., Osunkoya, B.O., Moore, D.L., Kemp, G.E., Fabiyi, A., 1972. A fatal human infection with Mokola virus. Am. J. Trop. Med. Hyg. 21, 959-963

Kimura, M., 1980. A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. J. Mol. Evol. 15, 111-120

King, A.A., Meredith, C.D., Thomson, G.R., 1993. Canid and viverrid viruses in South Africa. Onderstepoort J. Vet. 60, 295-299

Knobel, D.L., Cleaveland, S., Coleman, P.G., Fevre, E.M., Meltzer, M.I., Miranda, E.G., Shaw, A., Zinsstag, J., Meslin, F., 2005. Re-evaluating the burden of rabies in Africa and Asia. Bull. World Health Organ. 83 (5), 1-13

Koprowkski, H., 1994. The mouse inoculation test. In: Meslin, F.-X., Kaplan, M.M., Koprowski, H (Eds). Laboratory techniques in rabies, 4th Edn. World Health Organization, Geneva, pp. 80-85

Kuzmin, I.V., Orciari, L.A., Arai, Y.T., Smith, J.S., Hanlon, C.A., Kameoka, Y., Rupprecht, C.E., 2003. Bat lyssaviruses (Aravan and Khujand) from Central Asia: phylogenetic relationships according to N, P and G gene sequences. Virus Res. 97(2), 65-79

Kuzmin, I.V., Hughes, G.J., Botvinkin, A.D., Orciari, L.A., Rupprecht, C.E., 2005. Phylogenetic relationships of Irkut and West Caucasian bat viruses within the lyssavirus genus and suggested quantitative criteria based on the N gene sequence for lyssavirus genotype definition. Virus Res. 111 (1), 28-43

Kuzmin, I.V., Niezgoda, M., Franka, R., Agwanda, B., Markotter, W., Beagley, J.C., Urazova, O., Breiman, R.F., Rupprecht, C.E., 2008a. Lagos bat virus in Kenya. J. Clin. Microbiol. 46 (4), 1451-1461

Kuzmin, I.V., Wu, X., Tordo, N., Rupprecht, C.E., 2008b. Complete genomes of Aravan, Khujand, Irkut and West Caucasian bat viruses with special attention to the polymerase gene and non-coding regions. Virus Res. 136 (1-2); 81-90

Kuzmin, I.V., Niezgoda, M., Franka, R., Agwanda, B., Markotter, W., Beagley, .JC., Urazova, O.Y., Breiman, R.F., Rupprecht, C.E., 2008c. Seroprevalence to West Caucasian bat virus in Kenyan bats. Emerg.Infect.Dis.14,1887-1889

Kuzmin, I.V., Mayer, A.E., Niezgoda, M., Markotter, W., Agwanda, B., Breiman, R.F., Rupprecht, C.E., 2010. Shimoni bat virus, a new representative of the Lyssavirus genus. Virus Res. 149 (2), 197-210

Lackay, S.N., Kuang, Y., Fu, Z.F. , 2008. Rabies in small animals. Vet. Clin. North. Am. Small. Anim. Pract. 38 (4), 851-ix

Lumlertdacha, B., Boongird, K., Wanghongsa, S., Wacharapluesadee, S., Chanhome, L., Khawplod, P., Hemachudha, T., Kuzmin, I., Rupprecht, C.E., 2005. Survey for bat lyssaviruses, Thailand. Emerg. Infect. Dis. 11 (2), 232-236

Markotter, W., Kuzmin, I.V., Rupprecht, C.E., Nel, L.H., 2009. Lagos bat virus virulence in mice inoculated by the peripheral route. Epidemiol. Infect. 137, 1155-1162

Markotter W., Kuzmin I., Rupprecht C.E., Randles J., Sabeta C.T., Wandeler A.I., Nel L.H., 2006a. Isolation of Lagos bat virus from water mongoose. Emerg. Infect. Dis. 12(2), 1913-1918

Markotter, W., Randles, J., Rupprecht, C.E., Sabeta, C.T., Wandeler, A.I., Taylor, P.J., Nel, L.H., 2006b. Recent Lagos bat virus isolations from bats (suborder Megachiroptera) in South Africa. Emerg. Infect. Dis. 12, 504-506

Markotter, W., van Eeden, C., Kuzmin, I., Rupprecht, C.E., Paweska, J.T., Swanepoel, R., Fooks, A.R., Sabeta, C.T., Cliquet, F., Nel, L.H., 2008. Epidemiology and pathogenicity of African bat lyssaviruses. Dev. Biol. Basel. Krager. 131, 972-977

Markotter, W., Kuzmin, I., Rupprecht, C.E. Nel, LH., 2009. Lagos bat virus virulence by the peripheral route. Epidemiol. Infect.. 137, 1155-1162

Nel, L., Jacobs, J., Jaftha, J., von Teichman, B., Bingham, J., 2000. New cases of Mokola virus infection in South Africa: a genotypic comparison of Southern African virus isolates. Virus Genes, 20 (2), 103-106

Nel, L.H., Sabeta, C.T., von Teichman, B., Jaftha, J.B., Rupprecht, C.E., Bingham, J., 2005. Mongoose rabies in southern Africa: re-evaluation based on molecular epidemiology. Virus Res. 109, 165-173

Nel, L.H., Markotter, W. 2007. Lyssaviruses. Crit. Rev. Microbiol., 33 (4): 301-324

Nel, L.H., Rupprecht, C.E., 2007. Emergence of lyssaviruses in the Old World: the case of Africa. Curr. Top. Microbiol. Immunol, 315,161-193

Nig, A.J., Walker, P.L., 2009. Overview, prevention and treatment of rabies. Pharmacotherapy 29 (10), 1182-1195

Noah, D.L., Drenzek, C.L., Smith, J.S., Krebs, J.W., Orciari, L., Shaddock, J., Sanderlin, D., Whitfield, S., Fekadu, M., Olson, J.G., Rupprecht, C.E., Childs, J.E., 1998. Epidemiology of human rabies in the United States, 1980-1996. Annals of Internal Medicine, 128: 922-930

Ngoepe, C.E., Sabeta, C., Nel, L., 2009. The spread of canine rabies into Free State province of South Africa: A molecular epidemiological characterization. Virus Res.142, 175-180

Paweska, J.T., Blumberg, L.H., Liebenberg, C., Hewlett, R.H., Grobbelaar, A.A., Leman, P.A., Croft, J.E., Nel, L.H., Nutt, L., Swanepoel, R., 2006. Fatal infection with rabies-related Duvenhage virus, South Africa. Emerg. Infect. Dis. 12 (12), 1965-1967

Pfukenyi, D.M., Pawandiwa, D., Makaya, P.V., Ushewokunze-Obatolu, U., 2007. A retrospective study of rabies in humans in Zimbabwe between 1992 and 2003. Acta. Tropica. 102, 190-196

Reynes, J-M., Molia, S., Audry, L., Hout, S., Ngin, S., Walston, J., Bourhy, H., 2004. Serologic evidence of lyssavirus infection in bats, Cambodia. Emerg. Infect. Dis. 10 (12), 2231-2234

Sabeta, C.T., Mansfield, K.L., McElhinney, L.M., Fooks, A.R., Nel, L.H., 2007a. Molecular epidemiology of rabies in bat-eared foxes (*Otocyon megalotis*) in South Africa. Virus Res. 129 (1), 1-10

Sabeta, C.T., Markotter, W., Mohale, D.K., Shumba, W., Wandeler, A.I., Nel, L.H., 2007b. Mokola virus in domestic mammals, South Africa. Emerg. Infect. Dis. 13, 1371-1373

Sabeta, C.T., Blumberg, L., Miyen, J., Mohale, D., Shumba, W., Wandeler, A., 2009. Mokola virus involved in a human contact (South Africa). FEMS Immunol. Med. Microbiol, 1-6

Swanepoel, R., 2004. Rabies, in: Coetzer, J.A.W., Tustin, R.C. (Eds.), Infectious diseases of livestock: with special reference to Southern Africa. Oxford University Press, Cape Town, pp1121-1184

Tamura K., Dudley J., Nei, M., Kumar, S., 2007. MEGA4: molecular evolutionary genetics analysis (MEGA) software version 4.0. Mol. Biol. Evol. 24, 1596-1599

The Medical College of Wisconsin, 2009. Rabies treatment protocol. Available at: http:/www.chw.org

Van Thiel, P.A.M., de Biez, R.M.A., Eftimov, F., Tepaske, R., Zaaije L., van Doornum, G.J.J., Schutten, M., Osterhaus, A.D.M.E., Majoie, C.B.L.M., Aronica, E., Fehler-Gardiner, C., Wandeler, A.I., Kager, P.A., 2009. Fatal human rabies due to Duvenhage virus from bat in Kenya: Failure of treatment with coma-induction, ketamine and antiviral drugs. PLOS Negl. Trop. Dis. 3 (7), e428

Von Teichman, B.F., Thomson, G.R., Meredith, C.D., Nel, L.H. , 1995. Molecular epidemiology of rabies virus in South Africa: evidence of two distinct virus groups. J. Gen. Virol. 76, 73-82

Van Zyl, N., Markotter, W., Nel, L.H., 2010. The evolutionary history of African mongoose rabies. Virus Res., 150 (1-2): 93-102

Warrell, M.J., Warrell, D.A., 2004. Rabies and other lyssavirus diseases. The Lancet 363, 959 - 969

Zulu, G.C., Sabeta, C.T., Nel, L.H.,2009. Molecular epidemiology of rabies: focus on domestic dogs (*Canis familiaris*) and black-backed jackals (*Canis mesomelas*) from northern South Africa. Virus Res. 140, 71-78