Epidemiol. Infect. (2008), **136**, 1270–1276. © 2007 Cambridge University Press
doi:10.1017/S0950268807009582 Printed in the United Kingdom

SHORT REPORT
Use of a molecular epidemiological database to track human rabies case histories in South Africa

P. COETZEE[1,2], J. WEYER[1,2], J. T. PAWESKA[2], F. J. BURT[2], W. MARKOTTER[1] AND L. H. NEL[1]*

[1] *Department of Microbiology and Plant Pathology, University of Pretoria, Pretoria, South Africa*
[2] *National Institute for Communicable Diseases, Private Bag X4, Sandringham, South Africa*

(*Accepted 30 August 2007; first published online 26 October 2007*)

SUMMARY

The KwaZulu Natal and Eastern Cape provinces of South Africa have experienced a serious dog rabies epidemic over the past three decades. Towards a better understanding of this epidemic, we have previously analysed nucleotide sequences of 142 rabies virus specimens that were obtained from these regions during 2003–2004 and provided a molecular description of the geographical distribution of rabies viral variants in the affected provinces. Here, as an extension, we studied five human cases that occurred during 2002–2003 and demonstrated the use of the sequence database in tracking unknown human rabies case histories. We were able to identify the geographical origin of viruses responsible for each human infection and in one case obtained evidence that suggested a non-bite transmission of rabies virus from an infected dog to a child. We argue for the value of this information in surveillance and epidemiological study and in the follow-up and management of potential exposures.

Canine rabies is endemic among domestic dogs in the South African provinces of KwaZulu Natal (KZN) and the Eastern Cape (EC) from where about 6–29 human rabies cases (mean 14 cases), most of whom are young children are reported annually [1]. Canine rabies is a relatively new disease in the Republic of South Africa (RSA) and two major epidemics have occurred among the local dog populations of the eastern coastal regions of the country during the last 50 years. The first of these epidemics entered and manifested in KZN during 1964. The epidemic was thought not to have reached the EC, and was eradicated by 1968 through mass vaccination and strict dog control [1, 2]. New evidence suggests that this epidemic did in fact reach the EC, where it has persisted ever since this period [3]. Following the elimination of rabies from KZN by 1968, a new epidemic emerged in the northern parts of this province in 1976. This epidemic coincided with the outbreak of civil war in bordering Mozambique and the fleeing of refugees across the international border with RSA (northern KZN). This second epidemic has proven to be intractable, despite the vigorous control measures that have been implemented by the South African Directorate of Veterinary Services [1, 2].

We have previously studied the molecular epidemiology of this radiating epidemic, concentrating on 142 rabies virus (RABV) specimens that were obtained from domestic dogs and livestock from the KZN and EC provinces between the calendar years 2003–2004 [3]. The study targeted a 592 nucleotide region that encompassed the carboxyl terminal domain of the glycoprotein (G, cytoplasmic domain) and the G–L intergenic region (L representing the

* Author for correspondence: Professor L. H. Nel, Department of Microbiology and Plant Pathology, University of Pretoria, 0001 Pretoria, South Africa.
(Email: louis.nel@up.ac.za)

downstream 'large' viral polymerase gene) that constitutes a highly variable portion of the RABV genome. This region is suited for distinguishing closely related RABV variants in localized geographic domains [3–5] and was successfully applied towards the identification of regional RABV variants that were cycling during the study period [3]. The resulting phylogenetic analyses demonstrated that RABV in the KZN and EC provinces could be divided into subfamilies, clusters and groups that were unique to these provinces and to specific localized geographical areas within these provinces. This study represented the most complete molecular description of the canid rabies epidemic in the affected provinces to date and the generated sequence database is likely to be useful in future surveillance and epidemiological investigations [3]. Such epidemiological data can, for example, be applied to monitor the expansion of the epidemic into unaffected regions along the eastern coast of the country and in the same way the effectiveness of control strategies may be monitored. We have used the database to retrace human case histories where a case history was either incomplete or absent. Five human RABV specimens recovered from children aged <14 years from the KZN and EC provinces during 2002–2003 were studied and the main regional and local variants responsible for each infection were identified. The resulting evidence in one of these cases also suggested a passive non-bite transmission of RABV from an infected dog to a young child.

During 2002–2003, 21 human cases of rabies were laboratory-confirmed in South Africa [6, 7]. Of these, four specimens from KZN (KZNhmSPU03.15, KZNhmSPU03.77, KZNhmSPU03.272, KZNhmSPU02.326) and one from the EC (EChmSPU03.48) were selected for further study (Table). Inclusion of these specimens was based on the availability of post-mortem brain specimens and partially completed case histories as documented by field epidemiologists and state pathologists on specimen submission forms that are available from the Special Pathogens Unit at the National Institute for Communicable Diseases (NICD, RSA). Brain specimens were sampled at autopsy and were stored at −20 °C in 50% glycerol-phosphate-buffered saline (PBS) without further passage. RNA extraction, RT–PCR, nucleotide sequencing and phylogenetic analysis was performed as previously described [3]. Phylogenetic analysis indicated that KZNhmSPU03.15, KZNhmSPU03.77, KZNhmSPU03.272, KZNhmSPU02.326 and EChmSPU03.48, clustered into viral clusters KZN/A/V1, KZN/A/V5, KZN/A/V3/Cl1, KZN/A/V6 and EC/A/V2, respectively (Fig. 1). Clustering of RABV into these groups was in most cases supported by bootstrap values above 70%. The approximate intrinsic sequence identity viruses belonging to each of the clusters varied from 99·3% to 99·9%, whereas the mean sequence divergence between these clusters ranged from 1·0% to 2·2%. The average sequence identity for the human RABV specimens to the clusters into which they grouped ranged from 99·94% to 99·99% (p distances calculated in MEGA 3.1 [8], data not shown). All of the identified sequence clusters correlated with the general geographical regions where the cases were reported from, namely Lower Umfolozi, Port Shepstone, Tugela Ferry, Port Shepstone, and regions surrounding Cofimvaba – in that order (Figs 2 and 3). It was also of interest to note that within each of the sequence clusters from KZN, one or more animal viruses (KZN/A/V1 = 20; KZN/A/V3/CL1 = 2; KZN/A/V5 = 1; KZN/A/V6 = 7) that shared 100% G–L sequence identity with the respective human viruses, could be identified.

Rabies persists as a significant human and veterinary public health problem in RSA and much of the developing world. Some of the reasons for this include competing public health priorities, limited resources for veterinary control measures, shortages and prohibitive costs of post-exposure biologics, poorly informed communities, inadequately trained health and veterinary staff and inaccessible health-care facilities [1]. Most victims of the disease only present to primary health-care providers when already in the terminal stages of the disease. Further, the majority of victims are children who because of their height, inquisitive nature, interest in animals and inability to protect themselves, are particularly vulnerable to bite exposures from domestic dogs, especially to the face and head. Such injuries are often associated with shortened disease incubation periods [1]. Exposure of young children can complicate the task of reconstructing case histories, which is an essential step towards identifying possible contact routes and for determining where appropriate measures such as information campaigns and vaccination of domestic dogs should be undertaken. Often, young children are unable to avoid potential risk situations, are unaware of potential exposures and are unable to effectively communicate such potential exposures. Phylogenetic analysis of generated nucleotide sequence data can prove useful for retracing human case histories in regions where variant distributions have been

Table. *Case histories of selected human infections which occurred in the KwaZulu Natal (KZN) and Eastern Cape (EC) provinces during the calendar years 2002–2003*

SPU number*	Age/ sex	District exposure occurred in	Grid ref. number†	Date and type of exposure	Post exposure prophylaxis received	Date of onset	Date of first admittance to hospital/clinic	Symptoms	Date of death	Additional case details	Genbank accession number
KZNhmSPU03.15	5/F	KZN, Lower Umfolozi	N9	Bitten by dog on left shoulder on 17 Dec. 2002	None	?	7 Jan. 2003	Photophobia, restlessness, confusion	8 Jan. 2003	Exposure appears to have taken place in the context of a localized outbreak among domestic dogs, with at least two other dogs known to have died of rabies in the area during the same time period	DQ841546
KZNhmSPU03.77	3/F	KZN, Port Shepstone	H17	Bitten by dog on the left arm in February 2003	None	22 Mar. 2003	22 Mar. 2003	Fever, agitation, confusion, psychosis, hallucination	23 Mar. 2003	Infected dog was known to have bitten other children, but no additional details are provided	DQ841548
KZNhmSPU03.272	12/M	KZN, Tugela Ferry	N10	Received a superficial bite with no bleeding from a dog on an unknown date	?	25 Sep. 2003	?	Confusion	2 Oct. 2003	No additional case details are provided	DQ841549
KZNhmSPU02.326	6/M	KZN, Port Shepstone	H18	Source of infection not confirmed, child did not report a dog bite to parents	None	9 Nov. 2002	12 Nov. 2003	Fever, vomiting, and extreme aggression, with the patient known to have bitten one or more hospital staff	12 Nov. 2002	Neighbour's dog died on 9 Nov. 2002 but was not tested for rabies. Large number of believed contacts including family members and classmates	DQ841423
EChmSPU03.48	13/M	EC, Cofimvaba	?	Bitten by dog in November 2002	?	?	11 Feb. 2003	?	15 Feb. 2003	No additional details are provided	DQ841547

* Laboratory reference numbers: human viruses which were obtained from the National Institute for Communicable Diseases (NICD, RSA) are named using an SPU (Special Pathogens Unit) designation.

† The approximate regions from where specimens were obtained, have been indicated by using a grid reference system, as implemented on the Allerton Regional Veterinary Laboratory (KZN) specimen submission forms.

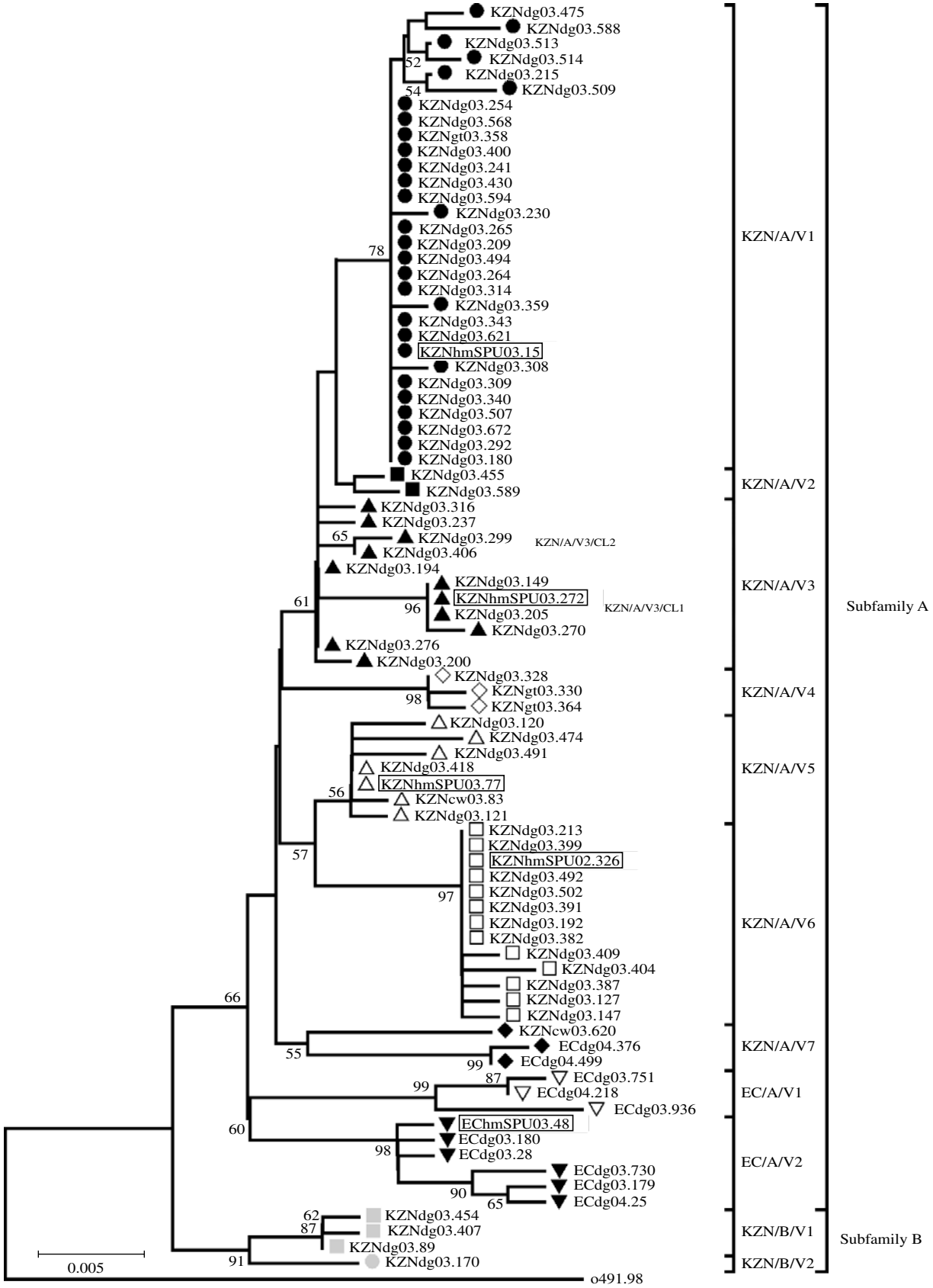

Fig. 1. For legend see following page.

https://doi.org/10.1017/S0950268807009582 Published online by Cambridge University Press

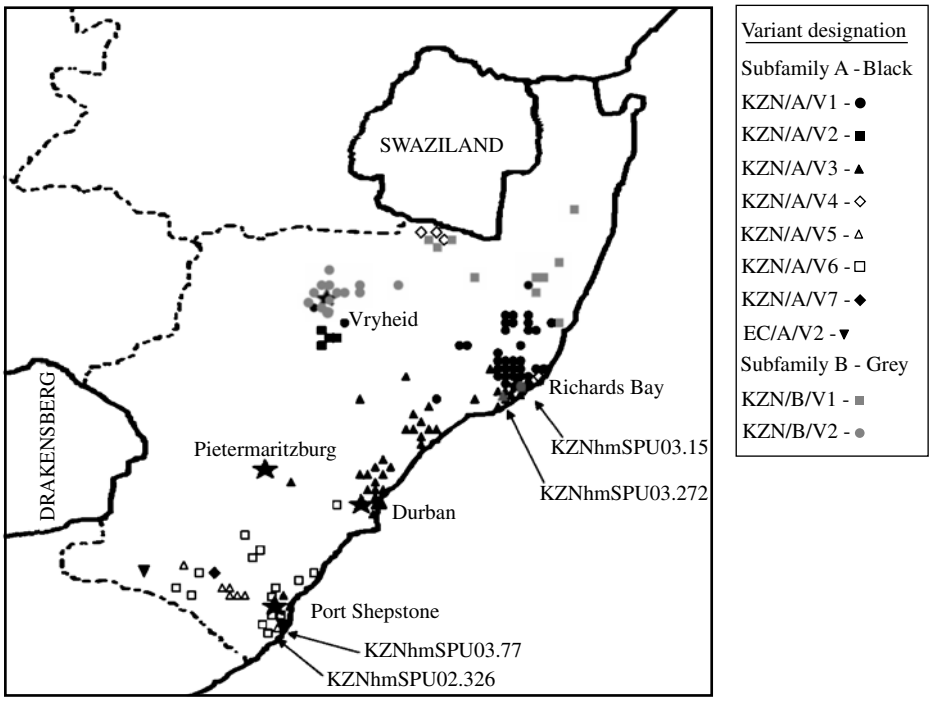

Fig. 2. A map of the KwaZulu Natal (KZN) province demonstrating the approximate geographic origin of canid rabies virus variants [3]. Symbols correspond to those used for the respective viral groupings on the phylogenetic tree in Figure 1. The approximate geographic regions where human cases originated from are indicated by arrows.

delineated and has in the past been applied to trace the importation of human rabies cases into non-endemic countries [9, 10], to trace the origin of cases in which there was no history of a bite exposure [11], and to identify foreign imported rabies cases that demonstrated extended incubation periods [9]. Without exception, the RABV studied here did conform to the phylogenetic grouping specific to viruses found to be circulating in the geographical domain associated with each case. Indeed it was found that human viruses in each of the respective KZN sequence clusters were identical to one or more animal viruses that were obtained from proximal geographic locations. This finding further highlights the fact that human exposures in the province occur in the context of highly localized outbreaks of specific RABV variants among domestic dogs. If this had not been the case, aspects of translocation or introduction of newly recognized variant(s) would have warranted further investigation.

Of particular interest is the case of a 6-year-old boy, case KZNhmSPU02.326. For this child there had been no history or evidence of a bite exposure or other close contact with any dog prior to him developing rabies. In retrospect, however, the next-door neighbour's dog had in fact died of unknown causes during the same relative time period, although rabies was not suspected nor considered in the animal. This unfortunate and injudicious oversight also meant that brain specimens were not available for our retrospective

Fig. 1. Neighborhood-joining tree of 82 nucleotide sequences of the cytoplasmic domain of the glycoprotein and G–L intergenic region, for selected canine, domestic livestock and human rabies viruses from KwaZulu Natal ($n=71$) and the Eastern Cape ($n=11$) provinces of South Africa [3]. Horizontal branch lengths are proportional to the similarity of the sequences within and between groups, with the scale indicating the amount of nucleotide sequence divergence in substitutions per site. The vertical lines are provided for purposes of clarity only. The cytoplasmic domain and G–L intergenic sequence region from a bat-eared fox specimens (o491.98) was used as reference sequence to root the tree, with virus numbers being preceded by a prefix indicating the geographic region (KZN, KwaZulu Natal; EC, Eastern Cape) as well as host species of isolation (dg, dog; cw, bovine; gt, caprine; hm, human). Symbols are used to denote subfamily and group divisions on the phylogenetic tree, as well as the geographical distribution of these variants on maps of the KZN and EC provinces in Figures 2 and 3. Human specimens are boxed in the phylogenetic tree.

https://doi.org/10.1017/S0950268807009582 Published online by Cambridge University Press

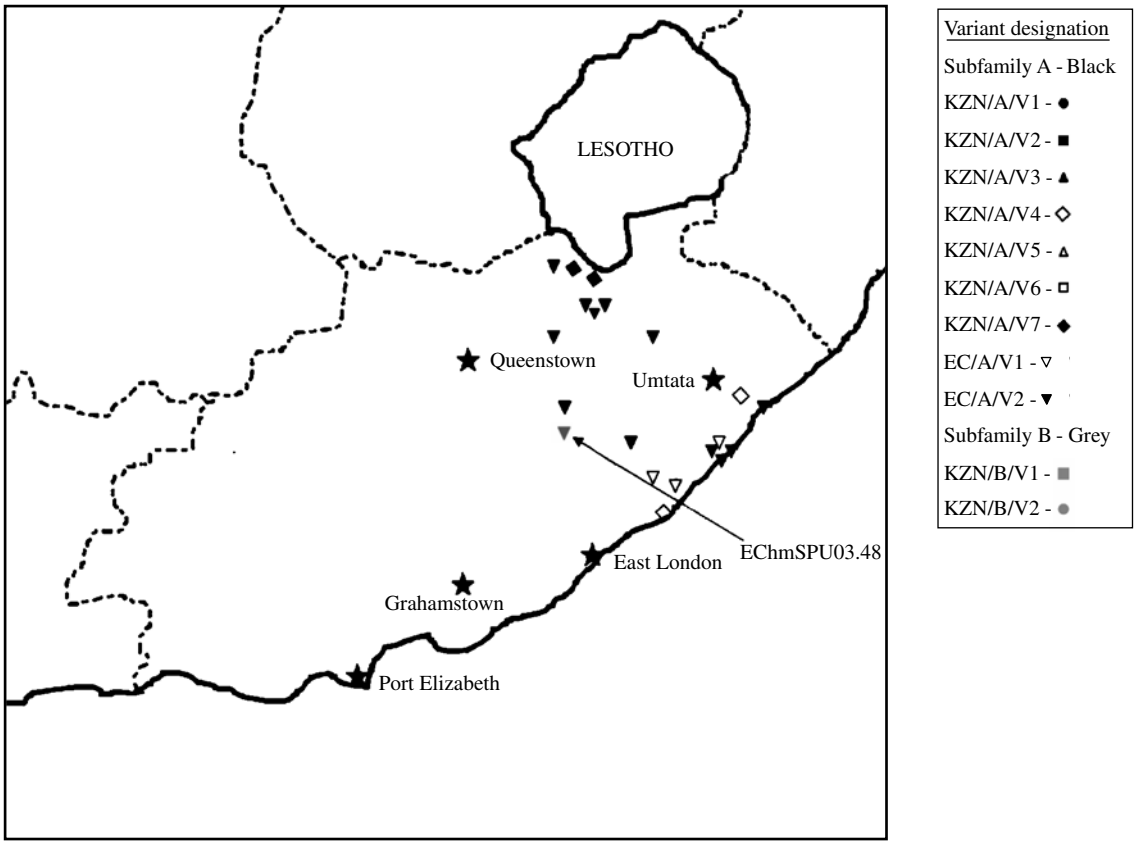

Fig. 3. A map of the Eastern Cape (EC) province demonstrating the approximate geographic origins of canid RABV variants [3]. Symbols correspond to those used for the respective viral groupings on the phylogenetic tree in Figure 1. The approximate geographic region where the human case originated from is indicated by an arrow.

analysis. Nevertheless, based on phylogenic identity to virus variants which circulated in the region where this exposure occurred, it is highly likely that a scratch or lick, or other exposure to saliva from this dog was responsible for the transmission of variant KZN/A/V6 to the child. Effective public health practices require that all rabies cases should be followed up and other potential exposures identified and managed. In cases such as these, where exposures go unnoticed, the use of molecular genetic tracking and locating of the source should be of particular importance if provided in a timely manner.

The KZN and EC provinces are endemic for the rabies-related viruses Lagos Bat virus (LBV) and Mokola virus (MOKV), while the mongoose biotype of classical rabies virus circulates among herpestids elsewhere in RSA [2, 5, 12]. Together with the recent isolation of LBV from a marsh mongoose in KZN, the possibility of potentially productive infectious cycles among terrestrial animals in these regions, which could lead to human exposures, has been raised [13]. From this perspective, our database as well as the methods that are described here should be continually expanded as new cases occur and should include not only canine rabies viruses, but indeed all the lyssaviruses of the region. Finally, rabies is also escalating in other parts of South Africa and the larger subcontinent – indeed a record number of human rabies cases were identified in South Africa in 2006 due to an outbreak among dogs in the Limpopo Province [14]. It is clear that rabies – a completely preventable disease – not only needs to be re-prioritized, but that improved tools can and should be applied within rabies control programmes.

ACKNOWLEDGEMENTS

This work was funded in part by a grant from the South African National Research Foundation (Gun 2054280/Pun 263880).

DECLARATION OF INTEREST

None.

https://doi.org/10.1017/S0950268807009582 Published online by Cambridge University Press

REFERENCES

1. **Bishop GC, *et al.*** Guide for the medical, veterinary and allied professions. Pretoria: Rabies Advisory Group, 2003; Government Printer (2nd printing).
2. **Swanepoel R.** Rabies. In: Coetzer JAW, Tustin RC, eds. *Infectious Diseases of Livestock with Special Reference to Southern Africa*. Cape Town, South Africa: Oxford University Press 2004, pp. 1123–1182.
3. **Coetzee P, Nel LH.** Emerging epidemic dog rabies in coastal South Africa: a molecular epidemiological analysis. *Virus Research* 2007; **126**: 186–195.
4. **Sabeta CT, Bingham J, Nel LH.** Molecular epidemiology of canid rabies in Zimbabwe and South Africa. *Virus Research* 2004; **91**: 203–211.
5. **Nel LH, *et al.*** Mongoose rabies in southern Africa: a re-evaluation based on molecular epidemiology. *Virus Research* 2005; **109**: 165–173.
6. **Anon.** National Institute for Communicable Diseases, Annual Report. Virology – Special Pathogens Unit, Rabies, 2002, pp. 76–77 (http://www.nicd.ac.za/#). Accessed 15 August 2007.
7. **Anon.** National Institute for Communicable Diseases, Annual Report. Virology – Special Pathogens Unit, Rabies, 2003, p. 61 (http://www.nicd.ac.za/#). Accessed 15 August 2007.
8. **Kumar S, Tamura K, Nei M.** MEGA3: integrated software for Molecular Evolutionary Genetics Analysis and sequence alignment. *Briefings in Bioinformatics* 2004; **5**: 150–163.
9. **Fooks AR, *et al.*** Risk factors associated with travel to rabies endemic countries. *Journal of Applied Microbiology* 2003; **94**: 1–6.
10. **Smith J, *et al.*** Case report: Rapid ante mortem diagnosis of a case of human rabies imported into the UK from the Philippines. *Journal of Medical Virology* 2003; **69**: 150–155.
11. **Messenger SL, Smith JS, Rupprecht CE.** Emerging epidemiology of bat-associated cryptic cases of rabies in humans in the United States. *Clinical Infectious Diseases* 2002; **35**: 738–747.
12. **Nel LH, *et al.*** New cases of Mokola virus infection in South Africa: a genotypic comparison of southern African isolates. *Virus Genes* 2000; **20**: 2, 103–106.
13. **Markotter W, *et al.*** Isolation of Lagos Bat Virus from water mongoose. *Emerging Infectious Disease* 2006; **12**: 1193–1918.
14. **Paweska J, Leman P, Blumberg L.** Rabies in South Africa, 2006. Sandringham, South Africa: National Institute for Communicable Diseases, 2007; *Communicable Diseases Surveillance Bulletin 7* (vol. 5, no 1).