Emergence of rabies in the Gauteng Province, South Africa: 2010–2011

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Canine rabies is enzootic throughout Sub-Saharan Africa, including the Republic of South Africa. Historically, in South Africa the coastal provinces of KwaZulu-Natal and Eastern Cape were most affected. Alarmingly, outbreaks of canine rabies have been increasingly reported in the past decade from sites where it has previously been under control. From January 2010 to December 2011, 53 animal rabies cases were confirmed; these were mostly in domestic dogs from southern Johannesburg, which was previously considered to be rabies free. In addition, one case was confirmed in a 26-month old girl who had been scratched by a pet puppy during this period. The introduction of rabies into Gauteng Province was investigated through genetic analysis of rabies positive samples confirmed during the outbreak period. In addition, the nucleotide sequences of incidental cases reported in the province for the past ten years were also included in the analysis. It was found that the recent canine rabies outbreak in the Gauteng Province came from the introduction of the rabies virus from KwaZulu-Natal, with subsequent local spread in the susceptible domestic dog population of southern Johannesburg. The vulnerability of the province was also highlighted through multiple, dead-end introductions in the past ten years. This is the first report of a rabies outbreak in the greater Johannesburg area with evidence of local transmission in the domestic dog population.

Introduction

Rabies is a fatal encephalitis caused by non-segmented RNA viruses belonging to the *Lyssavirus* genus, family Rhabdoviridae. Although rabies in dogs has been successfully controlled in several areas it is still a primary public health concern in many African, Asian and South American countries.

Canine rabies (also referred to as canid rabies) was introduced into the Republic of South Africa on several occasions, but most significantly in 1976. Since then the disease has been endemic in the coastal provinces of KwaZulu-Natal (KZN) and Eastern Cape (EC) (Swanepoel 2004). In 2005, a marked increase in the number of canine rabies cases was reported in Limpopo Province (LP), this coincided with the laboratory confirmation of 22 human rabies cases (Cohen *et al.* 2007). Since 2008, canid rabies has also re-emerged in Mpumalanga Province, with the disease radiating northwards and affecting areas where it was previously under control (Mkhize *et al.* 2010). Apart from rabies in domestic dogs, certain wildlife species also contribute to the epidemiological cycle of rabies in South Africa. The black-backed jackal (*Canis mesomelas*) and bat-eared fox (*Otocyon megalotis*) are reservoirs for the canid rabies biotype in the northern and south-western parts of South Africa respectively. The yellow mongoose (*Cynictis penicillata)* and other herpestids maintain the 'mongoose' biotype (commonly referred to as 'mongoose rabies') across the central plateau of South Africa (Nel *et al.* 2005; Swanepoel 2004). Human rabies cases are confirmed annually in South Africa and the majority of cases are linked to domestic dog exposures, this emphasises the importance of controlling the disease in dogs in order to prevent human cases (Weyer *et al.* 2011).

Rabies has occasionally been reported in Gauteng Province (GP) during the past ten years. Cases have been associated with the introduction of the disease from other areas of the country or have involved cases in wildlife in the more rural outlying areas of GP (Cohen *et al.* 2007; Weyer *et al.* 2011). These cases appeared to be isolated and were reportedly not associated with any sustained transmission. On 07 June 2010, rabies was confirmed in a domestic dog from Witpoortjie, Roodepoort. The dog had received veterinary treatment for two weeks and presented with symptomatology typical of rabies, including a sagging jaw, anxiety, hypersalivation and fits when it died. This dog apparently had no history of vaccination against rabies, had lived in the area for many years and, according to the owner, did not have any apparent contact with other animals. Within two weeks, two further dog cases were confirmed within a 3 km radius of

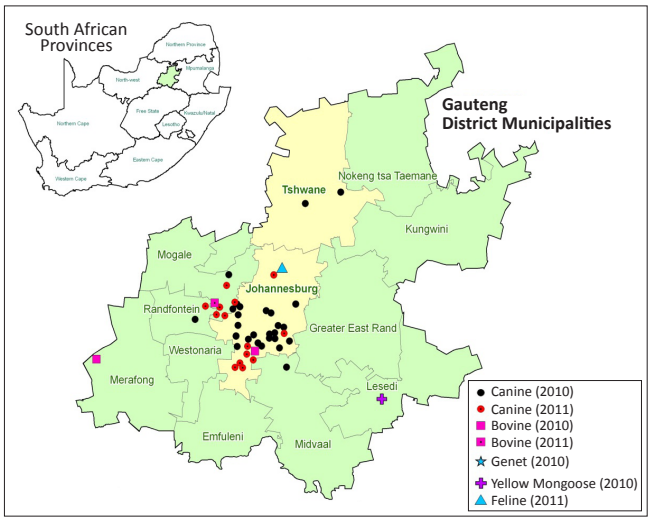
this case. Seven weeks after the third case was confirmed, a fourth was confirmed 15 km away in an easterly direction. Two weeks later, another case was identified approximately 15 km south-south-west of the fourth case. In this case the parents had died 2 weeks previously; an animal welfare veterinarian diagnosed tick bite fever (*Babesia* spp.). Thereafter the outbreak spread east, south and eventually west around the southern edge of Soweto. The spread of the outbreak appeared to be related to open, semi-rural corridors (for example, associated with mining activity) around densely populated areas. A total of 53 positive cases were confirmed throughout the outbreak period, with the majority of cases (n = 46, 86.8%) being directly associated with local transmission in the suburbs of southern Johannesburg, spanning an area 27 km north to south and 34 km east to west (Figure 1).

In order to establish the origin of this rabies outbreak and identify the introduction of rabies to GP, the G-L intergenic region of the rabies virus genome was sequenced and analysed from specimens collected from dogs and one human case from GP during the period 2000 to 2011 (Cohen *et al.* 2007; Coetzee & Nel 2007; Mkhize *et al.* 2010; Nel *et al.* 2005).

Materials and methods

Specimens, isolates and primary rabies laboratory diagnosis

A total of 309 brain specimens originating from dogs (n = 205), bovines (n = 15), cats (n = 42), mongooses (n = 20), jackal species (n = 3), unidentified bat species (n = 17) and other unidentified host species (n = 7) from GP, were submitted to the Agricultural Research Council - Onderstepoort Veterinary Institute (ARC-OVI) between January 2010 and December 2011 for laboratory confirmation of rabies (or lyssa) virus infection. A brain specimen was submitted to the National Institute for Communicable Diseases of the National Health Laboratory Service (NICD-NHLS) for one suspected human rabies case from Soweto during this time. The fluorescent antibody test (FAT) was prepared on brain impression smears using a fluorescein labelled polyclonal anti-lyssavirus conjugate (ARC-OVI, South Africa) as described elsewhere (Dean, Abelseth & Atanasiu 1996). A nuchal biopsy was also received for the suspected human case and tested using a real time reverse transcription polymerase chain reaction assay, as described elsewhere (Coertse *et al.* 2010).



Source: This figure was adapted from Dr Roy Williams (Onderstepoort Veterinary Institute)

FIGURE 1: Distribution of confirmed animal rabies cases for 2010–2011 outbreak in Gauteng Province.

Amplification, nucleotide sequencing and phylogenetic analysis

For phylogenetic analysis, the hypervariable region of the cytoplasmic domain of the rabies virus glycoprotein and the G-L intergenic region were targeted as before (Cohen *et al.* 2007; Coetzee & Nel 2007; Mkhize *et al.* 2010; Nel *et al.* 2005). A panel of 54 original brain specimens from the outbreak period in GP (n = 45), together with cases from GP for 2000–2009 (n = 9) were selected (Table 1). In addition, 19 sequences representing existing epidemiological cycles of rabies in South Africa, and available in the public domain, were used for the comparative genetic analysis (Genbank, NCBI) (Coertse *et al.* 2010; Nel *et al.* 2005; Ngoepe, Sabeta & Nel 2009; Sabeta, Bingham & Nel 2003; Swanepoel 2004; Weyer *et al.* 2011). Briefly, total RNA was extracted from the brain specimens using Trizol® reagent (Invitrogen, USA), a G-L reverse transcription polymerase chain reaction (PCR) was performed and sequencing reactions were prepared as described before (Coetzee & Nel 2007; Cohen *et al.* 2007; Mkhize *et al.* 2010; Nel *et al.* 2005). The consensus nucleotide sequences were assembled by comparing the forward and reverse sequences and phylogenetic analysis was undertaken as previously described (Coetzee & Nel 2007; Cohen *et al.* 2007; Mkhize *et al.* 2010; Nel *et al.* 2005).

Results

Laboratory confirmation of rabies cases

In total, 53 positive rabies cases (2010, n = 36 and 2011, n = 17) were confirmed from GP for the period January 2010 to December 2011 (representing a rate of positivity of 16.5%). The majority of these were from domestic dogs (n = 46, 86.7%) and the remaining cases were from bovines (n = 3, 0.01%) and one each from an unidentified mongoose, a banded mongoose (*Mungos mungo*), a small spotted genet (*Genetta genetta*) and a domestic cat (*Felis catus*). This rabies outbreak was also associated with one confirmed human rabies case that was reported in October 2010. The latter was confirmed by FAT on a brain specimen, but also RT-PCR on a nuchal biopsy.

Nucleotide sequence and phylogenetic analyses

Sequence analysis demonstrated that all of the outbreak virus cases clustered with the canid rabies biotype. Furthermore, the sequences clustered with previously characterised rabies viruses from dogs from KZN, with a statistically significant bootstrap support value of 97% (Figure 2). The mean sequence identity of the viruses in the KZN clade was 100%, not only confirming the compactness of the canid rabies

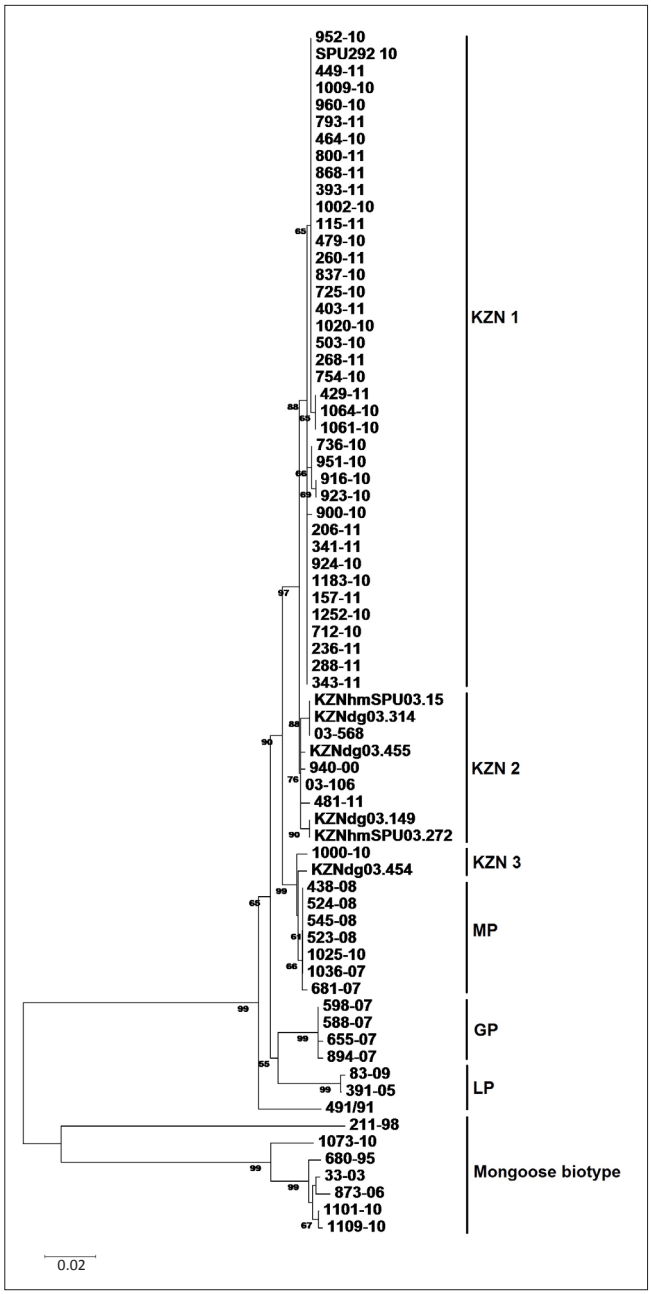
TABLE 1: A panel of 54 original brain specimens from the outbreak period in Gauteng Province.

Reference number	Date	Species of origin	Origin	Co-ordinate	GenBank	Reference
491/91	1991	Canine	Kempton Park, JHB, GP	28°14′ S, 26°04′ E	JF327499	This study
680/95	1995	Cape fox	Kroonstad, FS	27°16′ S, 27°31′ E	AY353996	Swanepoel 2004
211/98	1998	Canine	Kuruman, NC9	22°33′ S, 26°28′ E	AF303065	Sabeta *et al.* 2003
940/00	2000	Canine	Kempton Park, JHB, GP	28°15′ S, 26°06′ E	JF327497	This study
33/03	2003	Canine	Vereeniging, GP	28°03′ S, 26°37′ E	JF327504	This study
03/106	2003	Canine	Umfolozi, KZN	-	DQ841429	Ngoepe *et al.* 2009
03/568	2003	Canine	Umfolozi, KZN	-	DQ841535	Ngoepe *et al.* 2009
KZNhmSPU03.15	2003	Human	Umfolozi, KZN	-	DQ841546	Weyer *et al.* 2011
KZNhmSPU03.272	2003	Human	Tugela Ferry, KZN	-	DQ841549	Weyer *et al.* 2011
KZNdg03.149	2003	Canine	Umvoti, KZN	-	DQ841839	Nel *et al.* 2005
KZNdg03.314	2003	Canine	Hlabisa, KZN	-	DQ841480	Nel *et al.* 2005
KZNdg03.454	2003	Canine	Nongoma, KZN	-	DQ841515	Nel *et al.* 2005
KZNdg03.455	2003	Canine	Vryheid, KZN	-	DQ841516	Nel *et al.* 2005
391/05	2005	Canine	Thohoyandou, LP	30°43′ S, 22°48′ E	EF686089	Coertse *et al.* 2010
873/06	2006	Canine	Potchefstroom, NW	-	JF327505	This study
588/07	2007	Canine	Derdepoort, PTA	28°23′ S, 25°35′ E	JF327503	This study
598/07	2007	Canine	Derdepoort, PTA	28°23′ S, 25°35′ E	JF327500	This study
655/07	2007	Jackal	Derdepoort, PTA	25°25′ S, 28°22′ E	JF327501	This study
681/07	2007	Canine	Nkomazi, KZN	31°34′ S, 25°41′ E	FJ842751	Coertse *et al.* 2010
894/07	2007	Jackal	PTA, GP	25°17′ S, 28°38′ E	JF327502	This study
1036/07	2007	Canine	Hectorspruit, MP	31°48′ S, 25°31′ E	FJ842753	Coertse *et al.* 2010
438/08	2008	Canine	Nelspruit, MP	-	FJ842757	Coertse *et al.* 2010
523/08	2008	Canine	Nelspruit, MP	31°25′ S, 25°52′ E	FJ842758	Coertse *et al.* 2010
524/08	2008	Canine	Nelspruit, MP	31°25′ S, 25°52′ E	FJ842759	Coertse *et al.* 2010
545/08	2008	Canine	Nelspruit, MP	31°25′ S, 28°52′ E	FJ842761	Coertse *et al.* 2010
83/09	2009	Canine	Alexandria, JHB, GP	28°09′ S, 26°12′ E	JF327498	This study
464/10	2010	Canine	Witpoortjie, JHB, GP	27°81′ S, 26°16′ E	JF327493	This study
479/10	2010	Canine	Witpoortjie, JHB, GP	27°82′ S, 26°16′ E	JF327492	This study
503/10	2010	Canine	Witpoortjie, JHB, GP	27°83′ S, 26°14′ E	JF327494	This study
712/10	2010	Canine	Sophiatown, JHB, GP	27°97′ S, 26°17′ E	JQ756127	This study
725/10	2010	Canine	Eldorado Park, JHB, GP	27°92′ S, 26°90′ E	JF327496	This study
736/10	2010	Canine	Kibler Park, JHB, GP	28°01′ S, 26°31′ E	JF327495	This study
754/10	2010	Canine	Dobsonville, JHB, GP	27°83′ S, 26°22′ E	JQ756128	This study
837/10	2010	Canine	Meredale, JHB, GP	27°98′ S, 26°27′ E	JQ756129	This study
900/10	2010	Canine	Eldorado Park, JHB, GP	27°90′ S, 26°29′ E	JQ7561130	This study
916/10	2010	Canine	Highlands, JHB, GP	28°08′ S, 26°14′ E	JQ756131	This study
923/10	2010	Canine	Highlands, JHB, GP	28°08′ S, 26°14′ E	JQ756132	This study
924/10	2010	Canine	Lenasia, JHB, GP	27°83′ S, 26°31′ E	JQ756133	This study
951/10	2010	Canine	Crown Gardens, JHB, GP	28°00′ S, 26°14′ E	JQ756134	This study
952/10	2010	Canine	Mondeor, JHB, GP	28°00′ S, 26°22′ E	JQ756135	This study
960/10	2010	Canine	Meredale, JHB, GP	27°99′ S, 26°25′ E	JQ756136	This study
1000/10	2010	Canine	Waverley, PTA, GP	28°26′ S, 25°70′ E	JQ756137	This study
1009/10	2010	Canine	Protea North, JHB, GP	27°83′ S, 26°26′ E	JQ756139	This study
1020/10	2010	Bovine	Nancefield, JHB, GP	27°90′ S, 26°30′ E	JQ756140	This study
1025/10	2010	Canine	Danville, PTA, GP	28°12′ S, 25°74′ E	-	This study
1061/10	2010	Canine	Bergbron, JHB, GP	27°95′ S, 26°16′ E	JQ756141	This study
1064/10	2010	Canine	Protea North, JHB, GP	27°83′ S, 26°27′ E	JQ756142	This study
1073/10	2010	Bovine	Fochville, Krugersdorp, GP	27°19′ S, 26°32′ E	JQ756143	This study
1101/10	2010	Cynictis penicillata	Heidelberg, GP	28°43′ S, 26°51′ E	JQ756144	This study
1109/10	2010	Canine	Mulbarton, JHB, GP	28°05′ S, 26°29′ E	JQ756145	This study
1183/10	2010	Canine	Finsbury, JHB, GP	27°65′ S, 26°20′ E	JQ756146	This study
1252/10	2010	Canine	Vlakfontein, JHB, GP	27°82′ S, 26°17′ E	JQ756147	This study
SPU272/10	2010	Human	Soweto, GP	-	HQ734810	This study
115/11	2011	Canine	Mindalore, Krugersdorp, GP	27°82′ S, 26°13′ E	JN227482	This study
157/11	2011	Canine	Lenasia, JHB, GP	27°85′ S, 26°38′ E	JN227483	This study
206/11	2011	Canine	Lenasia, JHB, GP	27°85′ S, 26°39′ E	JN227484	This study
236/11	2011	Canine	Lenasia, JHB, GP	27°85′ S, 26°38′ E	JN227485	This study
260/11	2011	Canine	Haddon, JHB, GP	28°03′ S, 26°26′ E	JQ756148	This study
288/11	2011	Canine	Ennerdale, JHB, GP	27°98′ S, 26°02′ E	JN227487	This study
341/11	2011	Canine	Zakariyya Park, JHB, GP	27°90′ S, 26°36′ E	JQ756149	This study
343/11	2011	Canine	Lenasia, JHB, GP	27°87′ S, 26°36′ E	JQ756150	This study
393/11	2011	Canine	Krugersdorp, GP	27°74′ S, 26°18′ E	JQ756151	This study
403/11	2011	Bovine	Krugersdorp, GP	27°76′ S, 26°15′ E	JQ756152	This study
429/11	2011	Canine	Eldorado Park, JHB, GP	27°89′ S, 26°29′ E	JQ756153	This study
449/11	2011	Canine	Krugersdorp, GP	27°76′ S, 26°15′ E	JQ756154	This study
481/11	2011	Feline	Craigavon, JHB, GP	27°99′ S, 26°01′ E	JQ756155	This study
793/11	2011	Canine	Greenhills, JHB, GP	27°70′ S, 26°15′ E	JQ756156	This study
800/11	2011	Canine	Tshepisong, Krugersdorp, GP	27°78′ S, 26°18′ E	JQ756157	This study
868/11	2011	Canine	Krugersdorp, GP	27°79′ S, 26°06′ E	JQ756159	This study

GenBank, GenBank Accession number
GP, $n = 45$; $n = 9$
JHB, Johannesburg; GP, Gauteng Province; MP, Mpumalanga; nd, not done; NA, not applicable; PTA, Pretoria; NW, North-West; KZN, KwaZulu-Natal; NC, Northern Cape.
GP for 2000–2009

biotype, but also suggesting a common rabies cycle for these cases. A case of rabies in a domestic cat (481/11) identified from Johannesburg in 2011 does not appear to be associated with the outbreak. Although this isolate also clustered with the viruses originating from KZN, it grouped with the KZN 2 group and not the KZN 1 group (groupings refer to those as described by Coetzee and Nel 2007). No specific history could be traced for this case. Likewise, a case of rabies in a puppy (1000/10 Genbank accession number [GAN] JQ756137) in Pretoria was also not associated with the outbreak as the animal was brought from KZN by its owner and fell ill a month after arriving in GP. This isolate also clustered with the KZN 3 group of isolates, which corresponds to the travel history provided for the case. In 2010, a puppy was brought from Mpumalanga to Pretoria (1025/10 not sequenced). The animal started showing signs and symptoms of rabies after arrival in GP.

Previous incidental cases of dog rabies from GP were associated with clusters from north and north-western LP in 1991 and 2009 (491/91 GAN JF327499 and 83/09 GAN JF327498) and from KZN in 2000 (940/00) (Coetzee *et al.* 2008; Coertse *et al.* 2011). A cluster of four cases involving two dogs (588/07 GAN JF327503 and 598/07 GAN JF327500) and two jackals (655/07 GAN JF327501 and 894/07 GAN JF327502) were identified from the Pretoria area. The two dog cases were from the same smallholding. The sequences derived from these four cases were most closely related to the isolates circulating in LP. Several cases of mongoose rabies were identified during the outbreak period (1073/10 GAN JQ756143, 1101/10 GAN JQ756144 and 1109/10 GAN JQ756145). These involved a bovine case from Krugersdorp and a yellow mongoose from Heidelberg respectively. One of the cases involved a domestic dog from Mulbarton (1109/10), an area affected by the outbreak of rabies located south of Johannesburg. No history could be confirmed for this case, but could possibly be explained by translocation of the animal and exposure elsewhere in South Africa. Mongoose rabies was also diagnosed in a dog in 2003 (33/03 GAN JF327504) from Vereeniging located on the southern periphery of GP.



Note: The phylogenetic tree was derived from nucleotide sequences of dogs from the outbreak, a 26-month old child (SPU 272/10) and previously characterised isolates from South Africa. Selected virus isolates (2010 & 2011) are indicated by laboratory reference number (see also Table 1). The other outbreak isolates were removed from the analysis as they had 100% sequence identity. Only bootstrap support values ≥70% are shown on the nodes. The scale bar depicts nucleotide substitutions per site.
KZN, KwaZulu-Natal province; LP, Limpopo province; NC, Northern Cape province; MP, Mpumalanga province.

FIGURE 2: Neighbour joining tree showing the genetic relationships of rabies viruses.

Discussion

In South Africa, canid rabies has commonly been reported in domestic dogs from KZN and EC, and in the past five years also from MP and LP. Incidental cases of rabies have been reported from GP since 2000, but these appeared to be isolated introductions from other provinces or associated with the mongoose rabies cycle in South Africa. Apart from one cluster of four cases from Pretoria in 2007, no sustained transmission of cases was detected before 2010. The 2007 cluster involved two domestic dogs from the same smallholding and a jackal from the vicinity. No additional cases could be identified beyond the four described here.

It is apparent that GP is highly vulnerable to the introduction of rabies from other regions in the country; this is probably due to its economic significance and high level of movement of people to and from the province. The recent dog rabies outbreak in GP has resulted from introduction of the rabies virus from KZN, with subsequent local spread in the susceptible domestic dog population of southern Johannesburg. During this outbreak, three other cases of rabies indirectly linked to this outbreak and acquired outside of GP were confirmed.

This is the first report of a rabies outbreak with local transmission from the greater Johannesburg area. The public health significance of rabies, especially in domestic

dogs in densely populated settings, is clearly highlighted by recognition of a human case during this outbreak. Alarmingly, rabies in domestic dogs has emerged and re-emerged in several localities in South Africa in the past five years (Ngoepe, Sabeta & Nel 2009). The disease was previously under control in these areas; this highlights the importance of continued and sustained control efforts and more importantly, prevention of disease outbreaks through vaccination of pets and enhanced public awareness.

Conclusion

The rabies outbreak in Gauteng underscores that infectious diseases and in particular, zoonoses, can emerge in areas where they have not been detected previously. This therefore calls for the need to eliminate rabies at the animal source in order to minimise spill over into humans. In order to bring the rabies outbreak under control and probably eliminate the disease completely, the need for human and veterinary partners in both public and private organisations to work together should be further emphasised. This investigation was therefore aimed at establishing the origin of the rabies outbreak in Gauteng province and the usefulness of a local rabies sequence database was demonstrated in pinpointing the probable source of the 2010 infection as KwaZulu-Natal province.

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Competing interests

The authors declare that they have no financial or personal relationship(s) that may have inappropriately influenced them in writing this article.

Authors' contributions

C.T.S. (Onderstepoort Veterinary Institute) was the project leader and together with J.W. (National Institute of Communicable Diseases) & J.P. (National Institute of Communicable Diseases) conceived and designed the experiments, J.W. and J.M (Onderstepoort). and D.M. (Onderstepoort) performed most of the experiments. B.P. (Onderstepoort Veterinary Institute) & C.T.S. analysed the data. C.T.S., J.W., L.B. (National Institute of Communicable Diseases), J.T.P. (National Institute of Communicable Diseases) & P.L. (National Institute of Communicable Diseases) wrote the paper. P.G. (Gauteng Department of Agriculture), J.W., (Gauteng Department of Agriculture), W.S. (Onderstepoort Veterinary Institute) described the animal rabies outbreak. J.W., L.B., P.L. & J.T.P. made a contribution of the human case.

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