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SHORT REPORT
Emergence of dog rabies in the Northern region of Israel

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SUMMARY

Between 1979 and 2000, foxes constituted the main reservoir of rabies in the Northern regions of Israel. Following the implementation of the fox-targeted oral vaccination programme (ORV) in 1998, rabies was eradicated from this area. Subsequently during 2004–2007, the biological and molecular characterization of the rabies isolates showed that stray dogs emerged as the main animal reservoir in Northern Israel while lower numbers of cases were reported in domestic animals. The virus isolates from foxes and dogs differed, in their molecular characterization, suggesting two distinct separate lineages. The transition from fox-mediated rabies to dog-mediated rabies is of great concern to public health because of the close contact between dogs and the human population.

Key words: Dog rabies, emergence, Northern Israel.

Dogs are the main animal vectors of rabies to both humans and animals in most of the developing countries of Asia, Africa and South America. Human mortality from endemic canine rabies was estimated to be 55 000 deaths per year in Asia and Africa [1]. Rabies is endemic in Israel; until 1979 dogs (*Canis familiaris*) and jackals (*Canis aureus*) constituted the main reservoirs while dogs were the most common vector from 1967 to 1977. Two actions resulted in a 62% decrease in the number of cases during the subsequent 10 years; (*a*) the mass poisoning of jackals throughout the country and (*b*) the compulsory vaccination of dogs which was initiated in 1957. From 1979 the major rabies vector changed from urban dogs to sylvatic foxes (*Vulpes vulpes*) and the total number of positive fox cases increased significantly [2]. During 1996–1997 there were three human rabies cases, but the vectors were not identified [3]. The rabies viruses isolated from the human cases were typed as those maintained by foxes at that time.

In 1998, as a result of the increasing prevalence of rabies in Israel, the Israeli Veterinary Authorities decided to implement an eradication programme based on oral vaccination (ORV) to eliminate the disease from wildlife in the Northern regions of the country (Golan Heights and Galilee). The vaccine used in the ORV programme was Raboral V-RG [Merial Ltd, Athens, GA, USA (US Vet License no. 298)] delivered by aerial distribution using small aircraft and helicopters. The initial ORV programme in 1998 was conducted over an area of 495 km^2 (14–19 doses/km^2) and was extended in 2002 to the whole Northern region (6700 km^2). Vaccine dispersal was performed biannually in spring and autumn. In 2003–2005, ORV was extended to cover the entire country. From 2006, baits were dispersed only once a year in autumn. The countrywide dispersal in 2006 showed similar efficacy to the excellent results that were achieved in the 2002–2004 eradication area [2].

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Table 1. *Rabies virus isolates detected in the Northern region of Israel between 1998 and 2007*

Year	Submission for diagnosis*	Total cases in North Israel	Dog		Fox		Other animals		Human vaccinated‡
			No.	%†	No.	%†	No.	%†	
1998	523	63	8	1·52	27	4·58	28	5·35	n.a.
1999	489	52	6	1·22	22	4·49	24	4·90	n.a.
2000	601	21	3	0·49	7	1·16	11	1·83	n.a.
2001	304	1	1	0·32	0	0	0	0	n.a.
2002	291	0	0	0	0	0	0	0	n.a.
2003	270	0	0	0	0	0	0	0	n.a.
2004	306	3	0	0	0	0	3	0·98	n.a.
2005	357	33	20	5·60	0	0	13	3·64	160
2006	293	9	6	3·00	1	0·34	2	0·68	35
2007	306	15	8	2·61	4	1·30	3	0·98	41

n.a., Not available.
* Submission of suspected animals from the Northern region of Israel.
† 100× no. rabies-positive cases/total cases submitted for diagnosis from Northern Israel.
‡ Rabies post-exposure prophylaxis.

Despite the elimination of fox rabies in the Northern regions of Israel, a new outbreak was seen recently in this region. We now report the epidemiological and molecular characteristics of the stray dog-mediated rabies virus that emerged recently in Northern Israel after ORV implementation. It is also apparent that this transition imposes a new threat to public health.

In October 2004 a cow (Nt1211) from Kibbutz Natur on the Golan Heights showed clinical signs and rabies was suspected; after the cow was destroyed samples were submitted for diagnosis to the Rabies Laboratory at the Kimron Veterinary Institute. The specimens tested positive for rabies by direct immunofluorescence (FAT) staining using FDI-conjugated monoclonal antibodies (Fujirebio Diagnostics Inc., Malvern, PA, USA) and conjugated polyclonal antibodies (Chemicon International, Temecula, CA, USA) according to the manufacturer's instructions. The virus was isolated by the intra-cerebral mouse inoculation test, using suckling mice and by propagation in tissue culture [4]. Total RNA for RT–PCR assay was extracted from infected brain tissue with TRI reagent (Molecular Research Center, Cincinnati, OH, USA) according to the manufacturer's instructions. An oligonucleotide primer pair designated G and L were used to amplify an 880-bp segment of the G–L intergenic region by RT–PCR assay as previously described [5]. The G(+) strand 5′-GAC TTG GGT CTC CCG AAC TGG GG-3′ primer was used for the polymerase reaction at positions 4665–4687 of the G gene sequence, and the L(–) primer 5′-CAA AGG AGA GTT GAG ATT GTA GTC-3′ at positions 5543–5566 of the polymerase encoding gene according to the numbering of the published Pasteur virus (accession no. M13215) [5].

The 880-bp PCR product was purified with a GenEluted™ agarose spin column (Sigma, Israel) and sequenced by two of the PCR primers G and L using an Applied Biosystems (Foster City, CA, USA) automatic sequencer. The nucleotide sequences were aligned with the Clustal X program. A phylogenetic tree of 469-bp fragment (accession nos. EU589958–EU590040, EU715371–EU715395) from the G–L intergenic region was constructed by the neighbour-joining method, with the distance calculated using the Kimura-2 parameter with the MEGA program (version 3.1). The reliability of the phylogenetic groupings was evaluated using bootstrapping with 1000 replicates, as previously described [6].

Table 1 shows that from 1998, when ORV was implemented, the prevalence of fox rabies decreased gradually in these regions, indicating that the main rabies reservoir in the Northern region of Israel switched from foxes for dogs. During 2001–2003, the efficacy of ORV against wildlife rabies was demonstrated, as only one case of rabies was detected in Northern Israel, in spite of the intensive rabies surveillance (Table 1). The change in species reported, following the success of the ORV programme directed against foxes, suggests an epidemiological shift. This is substantiated by the molecular study. Phylogenetic analysis showed that seven rabies clades circulated in Israel [6]. The molecular analysis of this study

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revealed that sample Nt1211 belonged to new Israeli clade VII that was identified on the Golan Heights (Fig. 1). The new clade was found to be distinct from the other Israeli clades I–VI that circulated prior to implementation the ORV programme throughout the country [7].

During 2005, from a total of 357 submitted samples we diagnosed 34 rabies cases (20 dogs, eight cows, two wolves and one badger). A total of 32 isolates originated in the Northern regions, of which 31 isolates belonged to the same new clade VIIA, while one cow (Ah8437), was found to belong to clade II.

From the 293 samples submitted during 2006 from Northern Israel, nine rabies isolates were attained (six dogs, one horse, one cow and one fox). The phylogenetic analysis of the rabies isolates showed two new variants diverged from variant VII, variant VIIB comprising of one isolate (dog Af1086) and variant VIIC composed of two isolates (dog My9069 and cow Yr5355). Four rabies virus isolates belonged to clade VIIA. Three isolates (fox Bu9934, dog Yo9445, horse Ks8381) belonged to clade IB.

From the 306 samples submitted throughout 2007, 15 cases from the Golan Heights and Galilee and were diagnosed positive for rabies (eight dogs, four foxes, one jackal, one badger and one cow). Phylogenetic analysis revealed the identification of a third isolate, jackal St8059, belonging to variant VIIC in Galilee. Two dogs (Zo7828, Sn8653) belonged to variant VIIB, while six rabies isolates on the Golan Heights belonged to variant VIIA. Four fox isolates on the Golan Heights belonged to clade IB. This clade composed of seven isolates (five foxes, one dog, one horse), showed different sequences from the IA variant that circulated on the Golan Heights prior to 1998 and was found to be closely related to fox Sm0034 which was isolated in 2002 near the border with Jordan.

Analysis of the G–L intergenic region sequences of Turkish rabies isolates indicated that clade VII of dog-mediated rabies showed close relationship to the Turkish dog rabies isolates T4, T6 and T7 (Fig. 1), which suggested an association between rabies viruses in Turkey and the newly emerged virus in Israel.

Several hypotheses can serve to explain the rabies outbreak emergence in Northern Israel and are listed as follows:

(1) *Penetration of infected stray dogs into Northern Israel across the borders*. In Turkey, Syria, Lebanon and Jordan rabies is principally a disease of domestic dogs, with occasional cases observed in domestic cattle, wild animals and humans [8–10]. Between 1995 and 2002, 14 human cases were reported in Lebanon [8] and a dog bite was identified as the source of exposure in the majority [9]. Identification of a rabid stone marten isolate (S19655) provided evidence of rabies virus circulating in wild animals in Southern Lebanon during 1997. Phylogenetic analysis revealed that this isolate belonged to variant IC.

Between 1997 and 2002, 24 fatal human case of rabies occurred in Syria. Phylogenetic analysis provides support that variant VII, responsible for these cases in dogs, was recently introduced to the area and had not originated in Israel.

Our hypothesis is that rabies outbreaks in stray dogs and foxes occurred in neighbouring countries and were the result of penetration of infected animals into the Northern regions of Israel.

(2) *Translocation of infected stray dogs by humans travelling into and out of the Golan Heights*. Based on epidemiological investigations, two cases might have been caused by translocation of dogs from the Golan Heights to the Upper Galilee region. The first case was a dog (Hg9553), translocated to Hatzor Haglilit, while the second case was a cow (Mv7627), found positive in the Galilee region, far removed from the border area that might have been infected by the translocation of an infected dog from the Golan Heights to this area during its incubation period. We recently described the tracing of the regional source of a rabies infection in an Israeli stray dog [11] using molecular and antigenic characterization to show that the dog had been moved from the south to the centre of Israel.

(3) *Difference in the efficacies of the fishmeal polymer bait Raboral V-RG vaccine in two rabies animal reservoirs*. Although the bait was taken and induced protection in wildlife foxes, it was not effective in dogs. The V-RG vaccine was only efficient in dogs when it was administered directly by mouth [12]. It appears that the mode of delivery of the vaccine influences the acceptance of baits by dogs. Recently, the vaccination efficacy of livestock guard dogs by means of the fishmeal polymer bait Raboral V-RG vaccine was tested in the Golan Heights [13], however, the success of the procedure was limited; only 28% of the dogs consumed the baits, similar to previous studies conducted in Tunisia and Egypt [14, 15]. In those studies artificial fishmeal-containing candidate baits for delivery of oral rabies vaccines were poorly

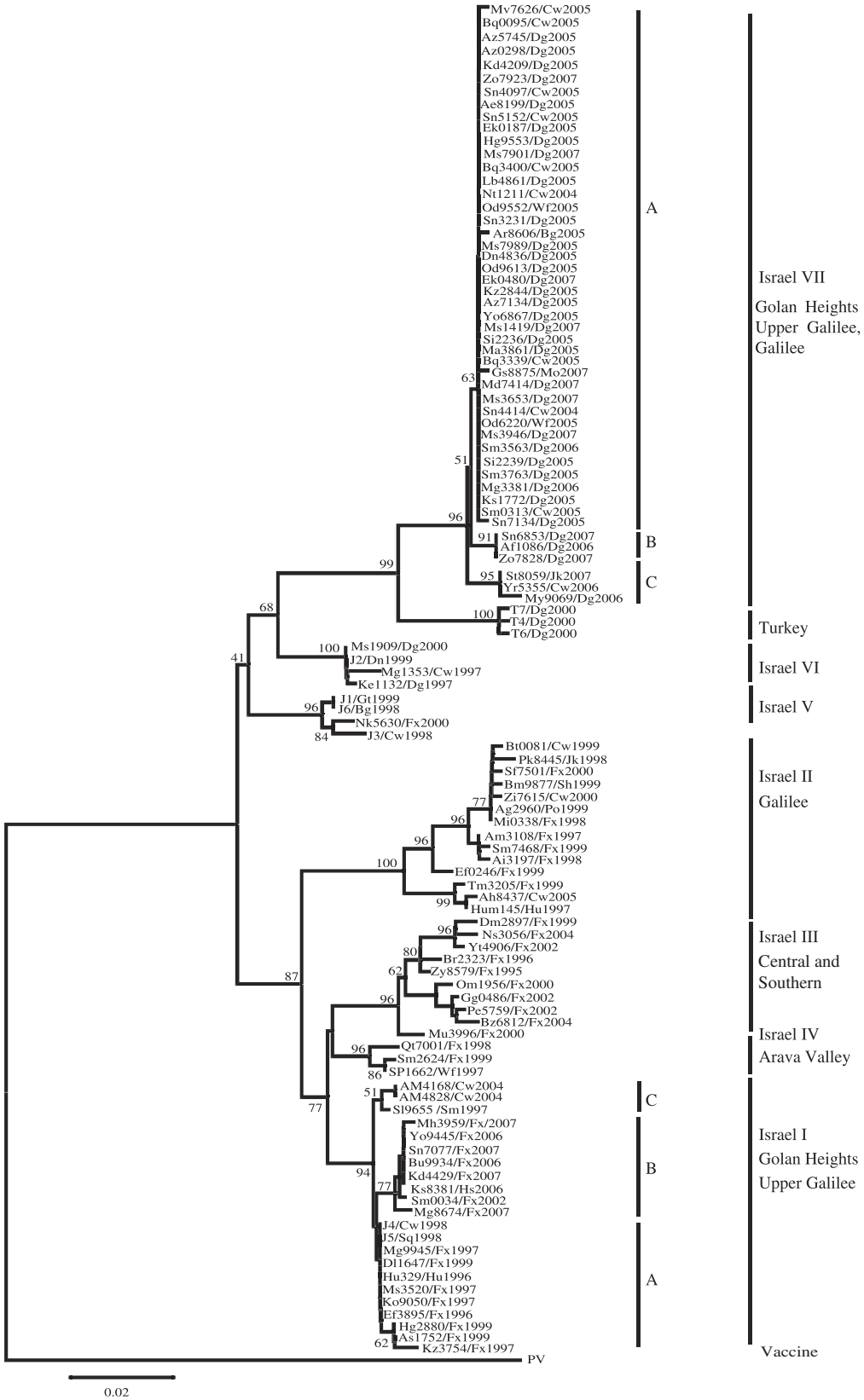

Fig. 1. Phylogenetic analysis of the Israeli, Turkish, Jordanian and South Lebanese rabies virus isolates (J, Jordan, SL, South Lebanon, T, Turkey). The animals and human isolates are indicated by abbreviated names: Dg, dog; Fx, fox; Cw, cow; Wf, wolf; Hs, horse; Dn, donkey; Gt, goat; Sh, sheep; Mo, mongoose; Sm, stone marten; Jk, jackal; Po, polecat; Bg, badger; Sq, squirrel; Hu, human. The scale bar indicates an evolutionary distance of 0·02 per position in the sequence.

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accepted by dogs. However, baits made from local material were found to have considerable advantages over imported manufactured baits [14, 15]. It seems that the ecological aspect of the bait content is important for the eradication of the rabies reservoirs formed by various wild animals.

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DECLARATION OF INTEREST

None.

REFFERENCES

1. **WHO Expert Consultation on Rabies.** World Health Organization. *Technical Report Series* 2005; **931**: 1–88.
2. **Yakobson BA, *et al.*** Rabies vaccination programme for red fox (*Vulpes vulpes*) and Golden Jackals (*Canis aureus*) in Israel (1999–2004). *Developments in Biology* 2005; **125**: 133–140.
3. **David D, *et al.*** Human rabies in Israel. *Emerging Infectious Disease* 1999; **5**: 306–308.
4. **David D, *et al.*** Rabies virus detection by RT–PCR in decomposed naturally infected brains. *Veterinary Microbiology* 2002; **87**: 111–118.
5. **Sacramento D, *et al.*** Molecular epidemiology of rabies in France: comparison with vaccine strains. *Journal of General Virology* 1992; **73**: 1149–1158.
6. **David D, *et al.*** Identification of novel canine rabies virus clades in the Middle East and North Africa. *Journal of General Virology* 2007; **88**: 967–980.
7. **David D, *et al.*** Molecular epidemiology of rabies virus isolates from Israel and other Middle and Near Eastern countries. *Journal of Clinical Microbiology* 2000; **38**: 755–762.
8. **Seimenis A, Morelli D, Mantovani A.** Zoonoses in the Mediterranean region. *Annual Institute Super Sanita* 2006; **42**: 437–445.
9. **Bizri AR, *et al.*** Human rabies in Lebanon: lessons for control. *Epidemiology and Infection*. 2000; **125**: 175–179.
10. **Kilic B, *et al.*** An important public health problem: rabies suspected bites and post-exposure prophylaxis in a health district in Turkey. *International Journal of Infectious Disease* 2006; **10**: 248–254.
11. **David D, *et al.*** Tracing the regional source of rabies infection in an Israeli dog by viral analysis. *Veterinary Record* 2004; **155**: 496–497.
12. **Rupprecht CE, *et al.*** Oral vaccination of dogs with recombinant rabies virus vaccines. *Virus Research* 2005; **111**: 101–105.
13. **Yakobson BA, *et al.*** Assessment on the efficacy of oral vaccination of livestock guardian dogs in the framework of oral rabies vaccination of wild canids in Israel. *Development of Biology* 2008; **131**: 257–264.
14. **Linhart SB, *et al.*** Acceptances of candidate baits by domestic dogs for delivery of oral rabies vaccine. *Onderstepoort Journal of Veterinary Research* 1997; **64**: 115–124.
15. **Matter HC, *et al.*** Test of three baits types for oral immunization of dogs against rabies in Tunisia. *American Journal of Tropical Medical Hygiene* 1995; **52**: 489–495.