

# Short Communications

## First reported cases of rabies in the Republic of Macedonia

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THE Republic of Macedonia is located in the southern region of the Balkan Peninsula. The country is bordered by Kosovo to the north-west, Serbia to the north, Bulgaria to the east, Greece to the south and Albania to the west. The area of the country is 25,713 km<sup>2</sup>, including around 80 per cent mountains and 20 per cent plains. The majority of Macedonia has a moderate continental climate with warm and dry summers and autumns, and relatively cold and wet winters. There had been no rabies cases reported in the country since 2000. Fig 1 shows the total number of rabies cases reported in 2010 in neighbouring countries. In those areas, the vector and reservoir of the disease is the red fox (*Vulpes vulpes*) which is responsible for more than 88 per cent of the reported cases (data extracted from Rabies Bulletin Europe). All countries (except Albania and Greece which are reported as

rabies-free) are currently enrolled in oral vaccination programmes which started in Kosovo in spring 2010. In Bulgaria, the first oral vaccination campaign was implemented in spring 2009 as well.

The first case of rabies in Macedonia was diagnosed on July 26, 2011, in a fox in a village (Stari Grad) south-west of Veles (Fig 1). A second case was identified in a fox on August 3, 2011, about 2 km south of the first one (Martolci village). These foxes entered the backyards of village houses during the day and attacked owned dogs. Two other cases were reported in the next month (on September 8 and 11) in wolves, in a village (Istibanja) around 5 km north of the city Vinica. The first wolf entered the backyard of a house and attacked a dog; the second animal was a young wolf approaching a house; the owner's dog barked, then the owner killed it with a hay fork.

All animals were sent to the rabies laboratory at the Faculty of Veterinary Medicine for testing. Rabies virus infection was diagnosed on brain smears and brain tissue by the direct fluorescent antibody test (Dean and others 1996) using a polyclonal antirabies conjugate (Bio-Rad) and by conventional PCR. The two foxes as well as the two wolves were found positive for rabies antigen/nucleic acid. Additional analysis, that is, cell isolation test and real-time reverse transcription qPCR were undertaken at the European Reference Laboratory for Rabies and provided similar positive results. Viral RNA from the four original infected brain tissues were extracted as described previously (Picard-Meyer and others 2004) and subjected to N gene amplification. cDNA synthesis was carried out with 5 µl of viral RNA and 20 pmol of random primer according to the manufacturer's instructions (Fermentas) using one RT cycle (50°C, 30 minutes). Real-time RT-qPCR was performed using 350 nmol each of primers N165-N146 and JW12 (Wakeley and others 2005), 2 µl of cDNA and the SsoFast Evagreen kit (Bio-Rad); one denaturation cycle (95°C, 30 seconds) followed by 40 amplification cycles (95°C, 5 seconds; 55°C, 30 seconds). PCR products (~589 bp) were sequenced by Beckman Coulter Genomics.

The samples from Macedonia were shown strongly positive by hnRT-PCR and by RT-qPCR (limit of detection of 1.3 copies/reaction) (Fig 2). The phylogenetic analysis against 64 reference sequences (neighbour-joining method, Mega version 5 software, Tamura and others 2011) showed that the four nucleoprotein sequences from Macedonia belong to the classical RABV lineage with a bootstrap value of 93 per cent (data not shown). Nucleoprotein sequence analysis (positions 71 to 747) between a Serbian isolate of 1997 (JF973785) and one Macedonian fox sample showed a perfect nucleotide similarity with 100 per cent identity (data not shown).

The EU provides financial support to both candidate and potential candidate countries through the Instrument for Pre-Accession Assistance, or IPA (Demetriou and Moynagh 2011). Financial assistance takes currently the form of national projects in seven Western Balkan countries (Albania, Bosnia-Herzegovina, Croatia, Montenegro, Kosovo, Republic of Macedonia and Serbia) for rabies and classical swine fever eradication. In August 2010, a two-year project named 'Capacity building of the veterinary service for implementation of EU Acquis' was started in Macedonia with an EC contribution of €1,919,764. The overall project objective is transposition of the EU Acquis, strengthening of inspection services, animal disease control and waste disposal as well as pig identification and registration. Regarding the rabies component, the project consists to elaborate a multiannual strategy of control (using oral vaccination of foxes), to improve passive surveillance and reporting as well as to enhance public awareness about the risks of this disease. Immunisation of foxes was initiated in spring 2011 and the second annual campaign was undertaken in autumn 2011.

Rabies surveillance is based on laboratory investigations of suspect animals for rabies diagnosis (Cliquet and others 2010). From 2000 to 2010, the level of rabies surveillance and public awareness was low, hence a few animals were analysed for rabies diagnosis. The number of samples analysed in Macedonia in 2009 and 2010 was 75 (including

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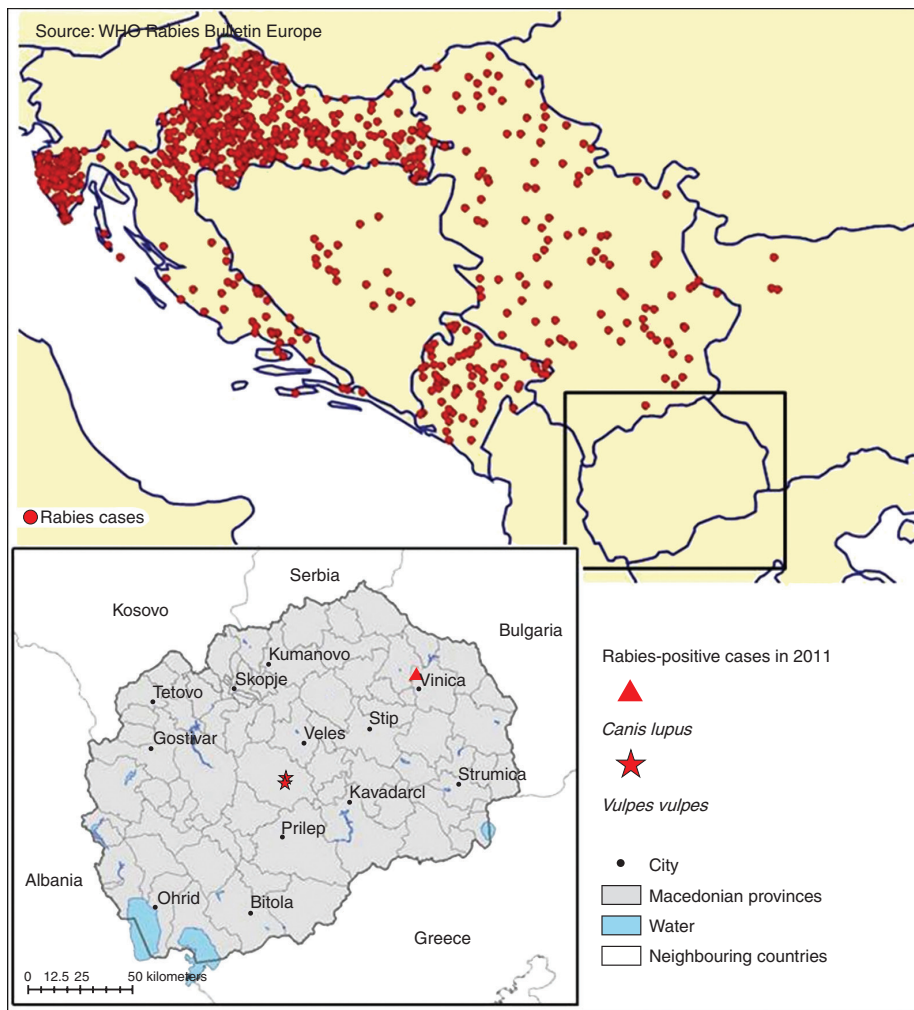


FIG 1: Map of Macedonia and neighbouring countries with distribution of rabies cases in 2010 and in October 10, 2011 (only for Macedonia)

53 wolves, 18 foxes and three dogs) and 49 (including 40 wolves and 4 foxes), respectively. In 2011, the number of samples tested until October 10, 2011 included 75 foxes, 33 wolves and two dogs (total of 110 tested animals). A significant increase in the number of animals submitted to rabies diagnosis was observed in 2011. The authors assume that the occurrence of sylvatic rabies was highly probable before 2011, considering rabies situation in neighbouring infected countries, and the fact that two cases were detected in the centre of the country. These four positive reports probably reflect a higher level of awareness of rabies among hunters and general public as well as an increased quality of the surveillance.

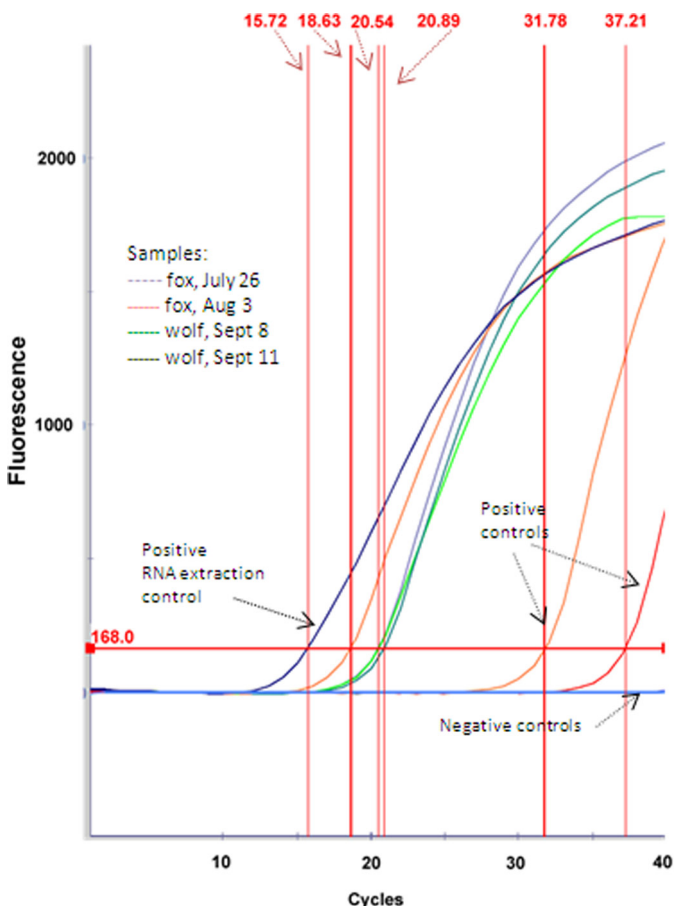
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FIG 2: Amplification of rabies virus (RV) nucleoprotein by RT-qPCR using RV-specific primers from pure genomic DNA generated from viral RNA extracted from RV-infected wolves and foxes from Macedonia



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