

Review Article

First Case Report of Rabies in a Wolf (*Canis Lupus Pallipes*) from India

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Abstract

This is the first documented evidence of rabies in the Indian wolf (*Canis lupus pallipes*) to date. The study describes the laboratory confirmation of rabies in wolf from the southern Indian state of Karnataka by the direct fluorescent antibody (DFA) test and a direct rapid immunohistochemistry test (dRIT). The PCR amplified products of complete N and G genes of this wolf RABV were sequenced and processed. The BLAST results and phylogenetic analysis revealed high homology and genetic relatedness of this RABV isolate with those from nearby geographic locations such as in India (human, buffalo), Pakistan (wolf, cattle) and Nepal (goat).

Analysis of the deduced amino acid sequences of the complete N and G proteins revealed that wolf RABV isolate recovered in this study belonged to the Arctic-like lineage, which is wide spread throughout the Indian subcontinent, and the region. These findings emphasise the necessity of epidemiological surveillance to characterize regional lyssaviruses to ascertain if wolves (and other wild species) serve as a significant population for RABV enzootic maintenance in India.

INTRODUCTION

Rabies has been reported in multiple species of wildlife in India, but there is no documented evidence of rabies in the Indian wolf (*Canis lupus pallipes*) to date. On 29 June 2013, a wolf entered two villages (2 km away from each other). The wolf bit 15 cattle in the first village, and then ran into a nearby village and bit 10 other cattle, 2 calves, one woman, and two men. The wolf was later trapped by the villagers and was killed. The head was transported to the Rabies Diagnostic Laboratory of the Veterinary College, Bangalore for laboratory diagnostic confirmation and RABV characterization (Figure 1). This paper describes the laboratory confirmation of rabies in this wolf from the southern Indian state of Karnataka and subsequent identification of the rabies virus (RABV) species by both N and G gene phylogenetic analysis.

MATERIALS AND METHODS**Laboratory confirmation by the direct fluorescent antibody (DFA) test and a direct rapid immunohistochemistry test (dRIT)**

Different sections of the brain, including the cerebrum, cerebellum, hippocampus, brain stem, as well as the salivary gland, were subjected to the Direct Fluorescent Antibody (DFA)

test, as suggested by the manufacturer (Light Diagnostics). In brief, the DFA was conducted by making tissue impression slides, dried at room temperature, fixed for 2 hr at -20 °C in acetone, and air dried at room temperature. The anti-N protein monoclonal antibody (Mab) – fluorescein isothiocyanate (FITC) conjugate (Light Diagnostics TM, Rabies DFA III, # 6500), with Evans blue (0.00125%) as the counter-stain, was added. Control slides using a Rabies Negative Control FITC conjugate (Light Diagnostics TM, # 5102) and Normal Goat FITC conjugate (Light Diagnostics TM, # 5202) were also prepared. The slides were incubated for 30 min at 37 °C in a humidity chamber. After staining, excess conjugate was removed and the slides were given a brief rinse under a stream of PBS, immersed and soaked in PBS for 3 min. Impression slides were blotted, air dried, mounted and read. In addition, a direct rapid immunohistochemistry (dRIT) technique was applied, as described [1]

Polymerase Chain Reaction (PCR) and phylogenetic analysis of N and G genes

The RNA was extracted from the brain stem by the Trizol® method, according to the manufacturer's recommendations (Invitrogen™). The cDNA was prepared by using a high capacity cDNA synthesis kit (Invitrogen™) and gene-specific primers, designated Lys 001 (for the complete N gene), MF 351 (for the

M-G gene segment), and 760 (for the G-L gene segment) for the complete G gene. The cDNA was further subjected to PCR using primers Lys 001 and Full R for the complete N gene, primer sets MF 351 and 989 for the M-G gene segment, and 760 & 308d for the G-L gene segment for the complete G gene amplification. The primers Lys 001, 989 and 308d were used as described by [2] The primer Full R was designed according to the RABV sequences available in GenBank and primers MF351 and 760 were designed based upon information by Dr. Natalia Kuzmina (CDC, Atlanta, GA). For PCR, initial denaturation was at 94°C for 1 min. followed by 40 cycles of amplification with each cycle comprising of denaturation at 94°C for 30 sec, annealing at 37°C for 30 sec and extension at 72°C for 1.30 min. The final extension was at 72°C for 10 min. The amplified products were electrophoresed in 1.5 Per cent agarose gel with ethidium bromide and the gel profile was documented (Bio rad, USA).

On elution from the gel, the complete N gene and M-G and G-L

segments of G gene amplicons were sequenced, aligned and the amino acid sequences of the complete N protein ORF (totally 449 amino acids) and amino acid sequences of the complete G protein ORF (totally 524 amino acids) were deduced. The deduced amino acid sequences were subjected to phylogenetic analysis using the Mega 5 program. The complete RABV N and G gene sequences available in Gen Bank from different parts of the world were included in the Multiple-sequence alignment after translation. Subsequently, comparisons were performed based on a Neighbor Joining-tree analysis.

RESULTS

Brain impression slides processed with anti-N protein Mab - FITC conjugate in the case of DFA (Figure 2) and anti-N protein Mab - biotin conjugate, in the case of dRIT (Figure 5) revealed the presence of viral inclusions in all portions of the brain, but not in the salivary glands (Figure 6). Further, control slides with either Rabies Negative Control FITC conjugate (Figure 3) or Normal



Figure 1 Head of Wolf (*Canis lupus pallipes*).

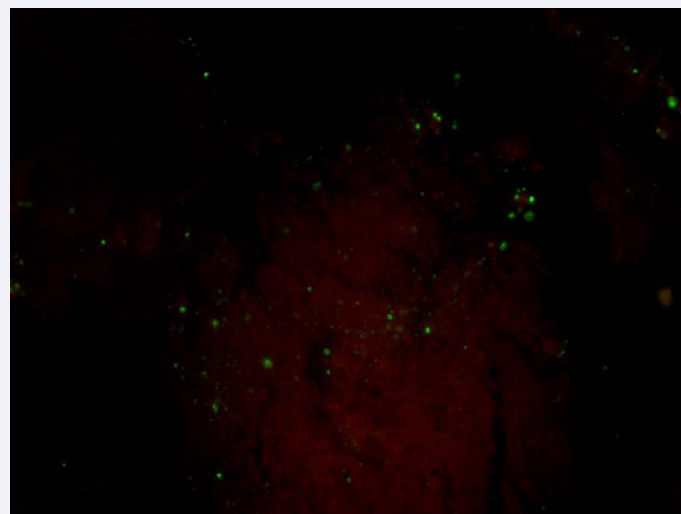


Figure 2 A DFA test of a brain stem impression from a rabies virus-infected wolf with anti-N protein Mab - FITC conjugate (Light diagnostics TM, Rabies DFA III, # 6500) showing viral inclusions (200X magnification).

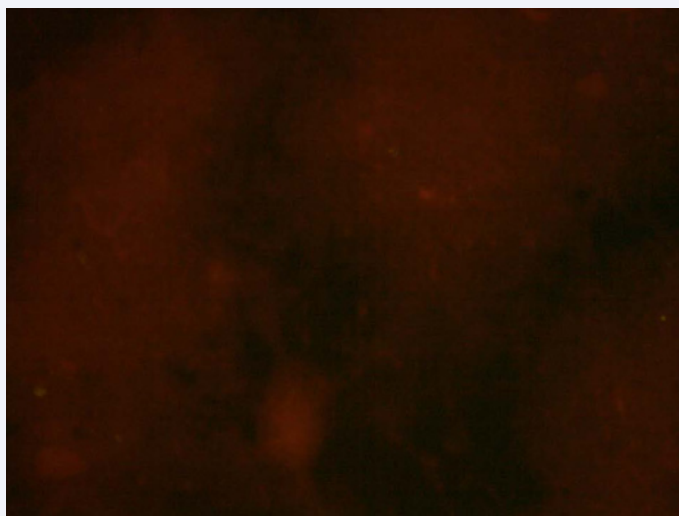


Figure 3 A DFA test of a brain stem impression from a rabies-virus infected wolf, with Rabies Negative Control FITC conjugate (Light diagnostics TM, # 5102) (200X magnification).



Figure 4 A DFA test of a brain stem impression from rabies virus-infected wolf, with Normal Goat FITC conjugate (Light diagnostics TM, # 5202) (200X magnification).

Goat FITC conjugate (Figure 4) did not reveal any apple-green fluorescence suggestive of RABV antigens.

The electrophoresis of PCR products revealed amplicons corresponding to the complete N (1511 bp), M-G segment (1431 bp) and G-L segment (1354 bp).

The BLAST analysis of complete N nucleotide sequences showed that the wolf RABV isolate recovered in the present study had a maximum N gene homology (97-100%) with a human (EF437215) and a buffalo (EF660245) RABV isolate of Indian origin and one bovine RABV isolate (HE802676) from Pakistan. Furthermore, the N gene amino acid based phylogenetic analysis of the wolf RABV isolate in this report clustered with the RABV isolates indicated above but also with an imported Indian clinical RABV isolate from Germany (AY956319), and two other Indian RABV isolates (EF611857, EF611862 - host species unknown) (Figure 7).

The BLAST analysis of complete G gene nucleotide sequences showed that the wolf RABV isolate in the present study had a maximum G gene homology (97-100%) with the same human (EF437215) RABV isolate from India, as observed for the N gene analysis, and a wolf (KC791852), and bovine (HE802675) RABV isolate of Pakistan origin and a goat (JX944593) RABV isolate from Nepal. Furthermore, the G gene amino acid based phylogenetic analysis revealed subclustering with a wolf (KC791852) RABV isolate from Pakistan; one human RABV isolate (EF437215) from India; and one goat RABV isolate (JX944593) from Nepal (Figure 8).

DISCUSSION

The DFA and dRIT on brain impressions of wolf confirmed rabies and the same was immediately communicated to the concerned. The bitten animals were provided immediate

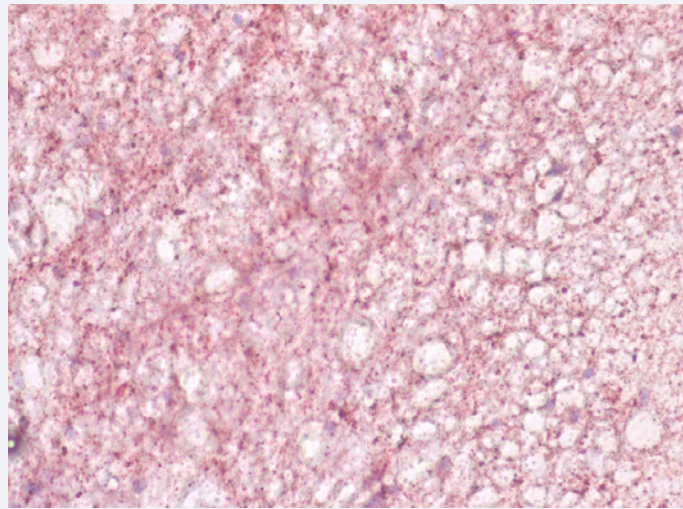


Figure 5 A dRIT test of a brain stem impression from a rabies virus-infected wolf, showing viral inclusions (200X magnification).

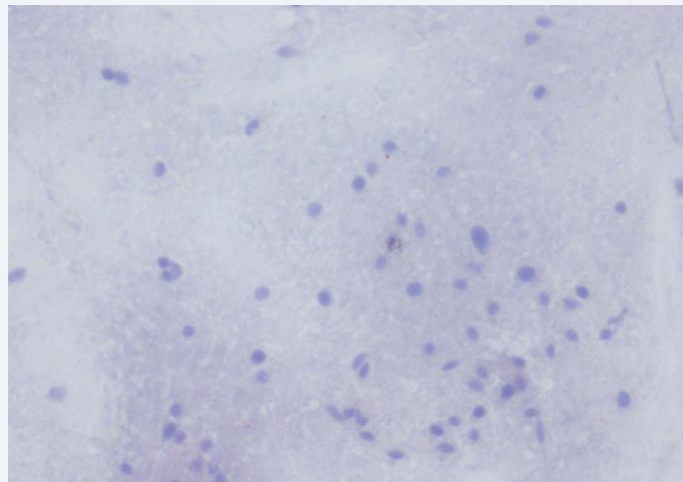


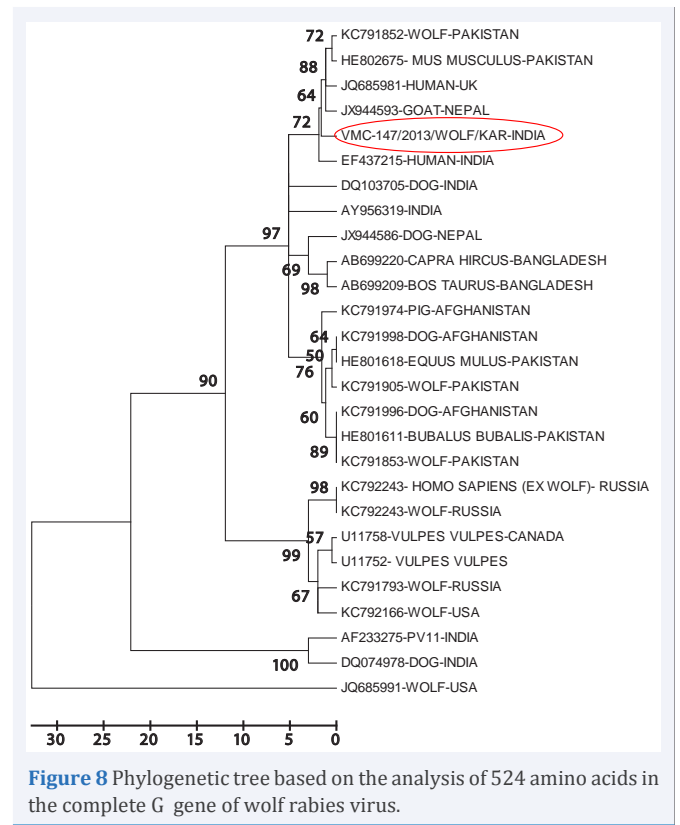
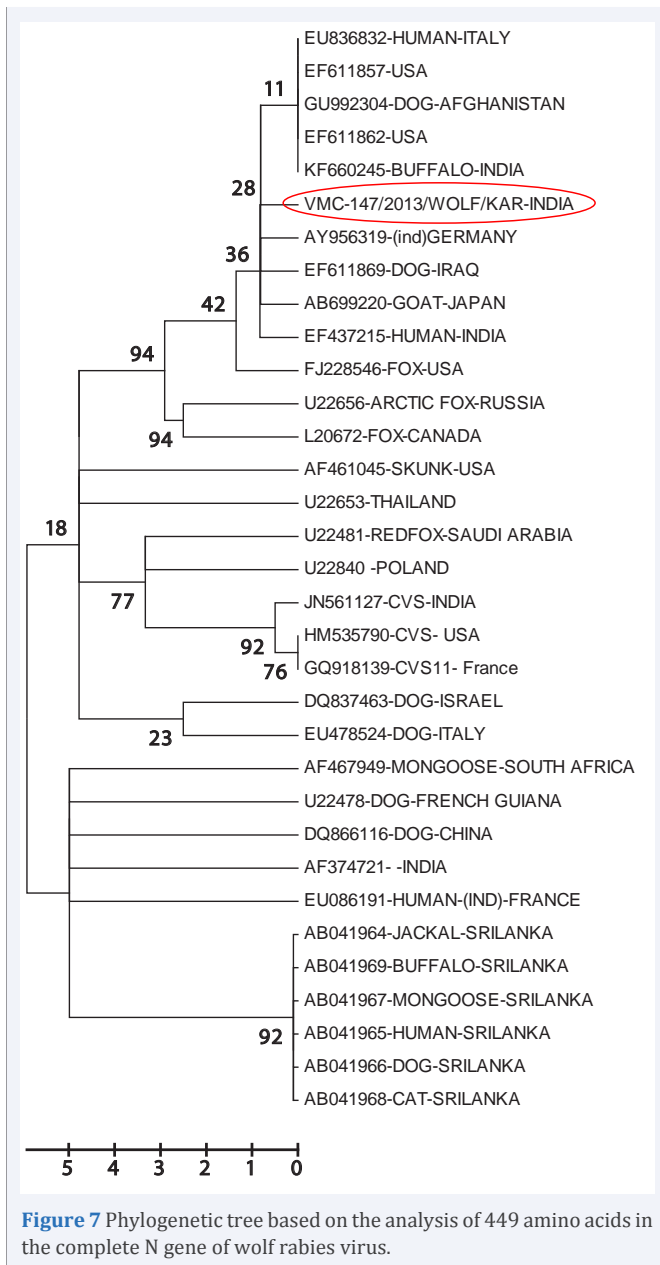
Figure 6 A dRIT test of a salivary gland impression from a rabies virus-infected wolf (200X magnification).

vaccination and human bite victims were administered post-exposure prophylaxis, including both vaccination and equine rabies immune globulin. The intramuscular vaccination regimen of 0, 3, 7, 14, 28 and 90 days was followed. All three human bite victims survived at the time of this communication. However, of 15 cattle bitten in the first village, 3 died after 3 months (post-vaccination regimen) showing typical clinical manifestations of rabies, whereas of 10 cattle bitten in the second village, one cow bitten in the eye died on the 14th day after the attack (post 0, 3 and 7 day vaccination) and two cattle died after 3 months (post vaccination regimen), exhibiting typical clinical signs of rabies. Of two bitten calves, one succumbed showing typical clinical signs of rabies after 4 months (post vaccination regimen). No post-mortem diagnostic analysis of these livestock was performed to confirm RABV infection.

The PCR based amplified products of complete N and G genes of RABV were sequenced and further processed. In general, the BLAST results and phylogenetic analysis based on both the N and G genes revealed high homology and genetic relatedness

of the RABV isolate recovered in this study, compared with RABV isolates from nearby geographic locations such as in India (human, buffalo), Pakistan (wolf, cattle) and Nepal (goat). This finding suggests that the genetic relatedness among the RABV isolates is ordered by geographic locations and less so by host species. Analysis of the deduced amino acid sequences of the complete N and G proteins revealed that the wolf RABV isolate recovered in this study belonged to the Arctic-like lineage, which is wide spread throughout the Indian subcontinent, and the region [3,4,5] Phylogenetic analysis with other isolates from wolves showed different RABV lineages, identified based on N or G gene comparisons. Two other RABV isolates from wolves in Israel (DQ837454, DQ837473) clustered with RABV isolates from the Middle East based on the N gene. Three other RABV isolates from wolves, two (KC792243, KC791793) from Russia and one (JQ685991) from the USA, clustered within the Arctic and SCSK lineages, respectively.

Despite the occurrence of rabies among wolves in Canada and the USA, no human fatalities have been reported to date [6-



9]. This is unlike the situation in Eurasia, where attacks of rabid wolves have resulted in some of the highest human case fatality incidents on record [4,10,11]. Human rabies postexposure prophylaxis is highly effective when intervention occurs in a timely and appropriate manner, such as combined with wound care, modern tissue culture vaccination, and the infiltration of rabies immune globulin. However, post exposure prophylaxis in naïve domestic animals is controversial, and has not been adequately documented. This observation is further evidence of the consideration for preventative pre-exposure vaccination to domestic species at risk, in which approved biologics have been licensed as pure, potent, safe, and efficacious [12].

In conclusion, this report describes the first laboratory confirmation of RABV in a wolf in India. This case suggests that RABV may be emerging in wolf populations in the southern part of India. If enzootic circulation occurs, this species could

serve as a significant vector for RABV transmission. Such an example illustrates the need for an enhanced nationwide epidemiological surveillance system in India to identify potential wildlife reservoirs outside of the typical canine host in India. As demonstrated in the multiple livestock cases, extension of local laboratory-based diagnostic services is required to confirm suspect cases based upon a history of exposure and onset of an acute, progressive encephalitis. Further, epidemiological surveillance is necessary to characterize regional lyssaviruses to generate the data needed to ascertain if wolves (and other wild species) are an accidental host, or serve as a significant population for RABV enzootic maintenance in India.

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REFERENCES

1. Dürr S, Naïssengar S, Mindekem R, Diguimbye C, Niezgodna M, Kuzmin I, et al. Rabies diagnosis for developing countries. *PLoS Negl Trop Dis*. 2008; 2: 206.
2. Kuzmin IV, Shi M, Orciari LA, Yager PA, Velasco-Villa A, Kuzmina NA, et al. Molecular inferences suggest multiple host shifts of rabies viruses from bats to mesocarnivores in Arizona during 2001-2009. *PLoS Pathog*. 2012; 8: 1002786.
3. Nadin-Davis SA, Turner G, Paul JP, Madhusudana SN, Wandeler AI. Emergence of Arctic-like rabies lineage in India. *Emerg Infect Dis*. 2007; 13: 111-116.

4. Jamil KM, Ahmed K, Hossain M, Matsumoto T, Ali MA, Hossain S, et al. Arctic-like rabies virus, Bangladesh. *Emerg Infect Dis.* 2012; 18: 2021-2024.
5. Pant GR, Lavenir R, Wong FY, Certoma A, Larrous F, Bhatta DR, et al. Recent emergence and spread of an Arctic-related phylogenetic lineage of rabies virus in Nepal. *PLoS Negl Trop Dis.* 2013; 7: 2560.
6. Chapman RC. Rabies: decimation of a wolf pack in arctic Alaska. *Science.* 1978; 201: 365-367.
7. Theberge JB, Forbes GJ, Barker IK, Bollinger T. Rabies in wolves of the Great Lakes region. *J Wildl Dis.* 1994; 30: 563-566.
8. Weiler GJ, Garner GW, Ritter DG. Occurrence of rabies in a wolf population in northeastern Alaska. *J Wildl Dis.* 1995; 31: 79-82.
9. Ballard WB, Krausman PR. Occurrence of rabies in wolves of Alaska. *J Wildl Dis.* 1997; 33: 242-245.
10. Janani AR, Fayaz A, Simani S, Farahtaj F, Eslami N, Howaizi N, et al. Epidemiology and control of rabies in Iran. *Dev Biol (Basel).* 2008; 131: 207-211.
11. Odontsetseg N, Uuganbayar D, Tserendorj Sh, Adiyasuren Z. Animal and human rabies in Mongolia. *Rev Sci Tech.* 2009; 28: 995-1003.
12. National Association of State Public Health Veterinarians, Inc. Compendium of animal rabies prevention and control, 2011. *MMWR Recomm Rep.* 2011; 60: 1-17.

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