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First report of GI.2 in a Cape hare (*Lepus capensis*) from Africa

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INTRODUCTION

Rabbit hemorrhagic disease virus (RHDV/GI.1) and European brown hare syndrome virus (EBHSV/GII.1) emerged around 1980 in China and in Sweden, respectively (Liu *et al*., 1984; Gavier-Widen D *et al*., 1989), and belong to "*Lagovirus europaeus*", member of the *Lagovirus* genus and *Caliciviridae* family (Le Pendu et al., 2017), causing acute and lethal forms of hepatitis. Despite their similarities, GI.1 and GII.1 are restricted to their natural hosts, European rabbit (*Oryctolagus cuniculus)* and European hare (*Lepus europaeus*) respectively (Lavazza *et al.*, 1996). In Tunisia, GI.1 was first described in 1993, while GII.1 has never been reported (Bouslama *et al*., 1996).

In 2010, a newly emerged RHDV genotype; RHDV2/b or *Lagovirus europaeus*/GI.2 was identified in France, and reached Tunisia in 2015 (Le Gall-Reculé *et al*., 2011; Chakroun *et al*., 2015). GI.2 was described to have distinctive phenotypic traits and specific antigenic profile. Indeed, GI.2 exhibits a larger host range, by infecting not only rabbits but also several hare species, including the European brown hare (*Lepus europaeus*), Cape hare (*Lepus capensis* subsp. *mediterraneus*), Italian hare (*Lepus corsicanus*) and mountain hare (*Lepus timidus*).

Here we report for the first time the identification of GI.2 in Cape hares (*Lepus capensis*) from Tunisia.

MATERIALS & METHODS

A Cape hare (*Lepus capensis*), was found dead in a forest area in the northeast of Tunisia, in the governorate of Nabeul (36°54'58.22"N, 11° 4'38.89"E) (Figure 1). Post mortem examination of the carcass revealed no external lesions with no signs of decomposition, but presented extensor rigidity (Figure 2). Internal gross lesions included hemorrhagic lesions in different organs including the liver, lungs and spleen (Figure 3).

Thirty-mg of liver sample was homogenized using a rotor-stator homogenizer at 30 Hz for 5 min. RNA was extracted using the GeneJET RNA Purification Kit following the manufacturer's protocol.

cDNA was synthesized with the NZY First-Strand cDNA Synthesis Kit according to the provided protocol. The obtained cDNA was used for PCR amplification with the primers U38F (5'-CAGCGGGCACTGCTACCACAGCATC-3') and EBHS9R (5'-CCAGCCCAACCAGCYTACAT-3'). The PCR product was purified and sequenced in an automatic sequencer.

A Blast analysis (https://blast.ncbi.nlm.nih.gov/Blast.cgi) was performed to confirm the identity of the sequence and a maximum likelihood (ML) phylogenetic tree was constructed in MEGA 5.1 software (Kumar et al. 2018).

FIG 2: A cape hare that died of GI.2 infection with typical rabbit RHD posture

FIG 3: Post-mortem findings Showing hemorrhagic lesions in different organs

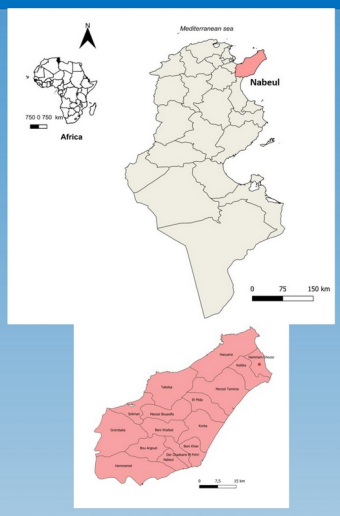

FIG 1: Map of Tunisia showing the sampled region

RESULTS

A partial sequence of the capsid gene was obtained for the lagovirus infecting the Cape hare (~300 bp). The Blast analysis revealed an identity >99% with strains identified previously as *Lagovirus europaeus*/GI.2 (e.g. Tunisian strains collected between 2015-2018, GenBank accession numbers MK629981, MK629984-7, MK629991). This confirms that the Cape hare was infected with GI.2.

The phylogenetic results, reported in Figure 4, clearly show that the GI.2 strain identified in the Tunisian Cape hare clusters with other GI.2 strains identified in rabbits, being more closely related to other Tunisian strains.

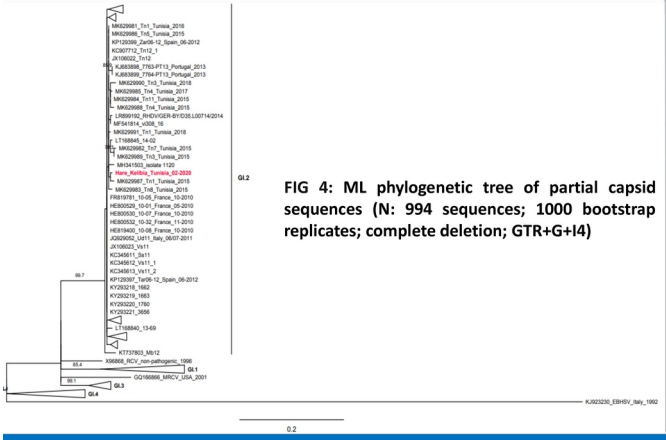

FIG 4: ML phylogenetic tree of partial capsid sequences (N: 994 sequences; 1000 bootstrap replicates; complete deletion; GTR+G+I4)

DISCUSSION

The present work reports the occurrence of GI.2/RHDV2 case in a Cape hare (*Lepus capensis*) from Tunisia, causing hemorrhagic lesions and necrotizing hepatitis, consistent with a lagovirus infection. The characterized strain was most closely related to those strains locally circulating in rabbits, suggesting that the reported case resulted from a spillover event from rabbit outbreaks in neighboring areas, and a strong epidemiological link between cases in rabbits and hares.

This is the first report of a GI.2 infection in *Lepus* species in Africa. Indeed, unlike GI.1, whose unique host is the European rabbit (*Oryctolagus cuniculus*), GI.2 can overcome the species barrier and cause severe disease in several hare species. In all reported cases, infected hares were found in regions where rabbits also succumbed to GI.2 infection, suggesting viral spillover from sympatric rabbits to hares.

Our findings have significant implications for Africa and highlight the need of virus surveillance, especially at the interface between wild and domestic leporids. Furthermore, it may have significant epidemiological and evolutionary implications in areas where hares are widespread, increasing the probability of recombination events between GI.2 and GII.1 as supported by the recent detection of GI.2/GII.1 recombination (Szillat et al., 2020).

In conclusion, demonstration that Tunisian Cape hare is susceptible to GI.2 further confirms its increased host range. A large-scale survey on older samples is needed to assess more precisely the date of the first crossing of species barrier in Tunisia and increased monitoring efforts will be required to explore the epidemiological circumstances of the outbreak and to further elucidate the role that hares play in the epidemiology of lagoviruses in Africa.

BIBLIOGRAPHIC REFERENCES

Liu SJ, Xue HP, Pu BQ, Qian NH. A new viral disease in rabbits. *Anim Husb Vet Med Xumu Yu Shouyi* 1984;**16**:253–5.

Gavier-Widen D. and Morner T. The European brown hare syndrome in Sweden. *Verhandlungsbericht uber Erkrankung der Zootiere.* 1989; **31**: 261–264

Le Pendu, J.; Abrantes, J.; Bertagnoli, S.; Guitton, J.S.; Le Gall-Recule, G.; Lopes, A.M.; Marchandeau, S.; Alda, F.; Almeida, T.; Celio, A.P., et al. Proposal for a unified classification system and nomenclature of lagoviruses. *The Journal of general virology.* 2017; **98**:1658-1666, doi:10.1099/jgv.0.000840.

Lavazza A, Scicluna MT, Capucci L. Susceptibility of hares and rabbits to the European brown hare syndrome virus (EBHSV) and rabbit haemorrhagic disease virus (RHDV) under experimental conditions. *Zentralblatt fur Veterinarmedizin Reihe B Journal of veterinary medicine Series B*. 1996; **43**(7):401-10.

Bouslama, A.; De Mia, G.M.; Hammami, S.; Aouina, T.; Soussi, H.; Frescura, T. Identification of the virus of rabbit haemorrhagic disease in Tunisia. *The Veterinary record* 1996; ***138***:108-110, doi:10.1136/vr.138.5.108.

Le Gall-Recule, G.; Zwingelstein, F.; Boucher, S.; Le Normand, B.; Plassiart, G.; Portejoie, Y.; Decors, A.; Bertagnoli, S.; Guerin, J.L.; Marchandeau, S. Detection of a new variant of rabbit haemorrhagic disease virus in France. *The Veterinary record* 2011, **168:**137-138, doi:10.1136/vr.d697.

Chakroun, C., et al. 'La nouvelle forme de la maladie hémorragique virale (VHD) en Tunisie due au virus variant', Bulletin d'information avicole et cunicole *(GIPAC).* 2015, *23* 56, 23-6.

Kumar, Sudhir, et al. 'MEGA X: Molecular Evolutionary Genetics Analysis across Computing Platforms', Molecular biology and evolution, 2018; **35** (6), 1547-49.

Szillat KP, Höper D, Beer M, König P. Full-genome sequencing of German rabbit haemorrhagic disease virus uncovers recombination between RHDV (GI.2) and EBHSV (GII.1). *Virus Evolution*. 2020; **6**(2).