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*Short communication*

# The genetic analysis of new Polish strains of European brown hare syndrome virus

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## Abstract

In this paper we describe recently occurring outbreaks of European brown hare syndrome (EBHS) in a captive hare population. The aim of our study was to evaluate the phylogenetic position of detected Polish strains compared to other European strains of EBHSV. Investigations were undertaken in hares from different provinces of Poland. Liver or spleen samples were tested for viral RNA using the RT-nested PCR method and the products were subsequently sequenced. The genetic analysis was based on the fragment of gene encoding viral capsid protein; it revealed a high homology and close relationship between Polish and European EBHSV strains isolated between 2001 and 2011.

**Key words**: European brown hare syndrome virus, genetic analysis

## Introduction

European brown hare syndrome virus (EBHSV) is classified within the *Lagovirus* genus of the *Caliciviridae* family. It causes a highly contagious and fatal disease in free-living and farmed hares. Since the early 1980s, the disease has been endemic in Europe. In Poland, EBHSV was detected for the first time in 1992 and the presence of the virus in the hare population was confirmed in the following years. The aim of the present study was the phylogenetic analysis of new EBHSV strains circulating in hares in Poland.

## Materials and Methods

In the spring months of 2011 high mortality of hares was observed in 4 private farms located in different provinces of Poland: Lubuskie, Warmia-Masuria, Lubelskie and Świętokrzyskie. Fragments of internal organs (liver or spleen) from 12 dead hares were collected during *post mortem* examination. The number of sampled animals from each province was as follows: 4 from Lubuskie, 2 – Lubelskie, 3 – Warmia-Mazuria and 3 – Świętokrzyskie. Extraction of the total RNA from organ homogenates,

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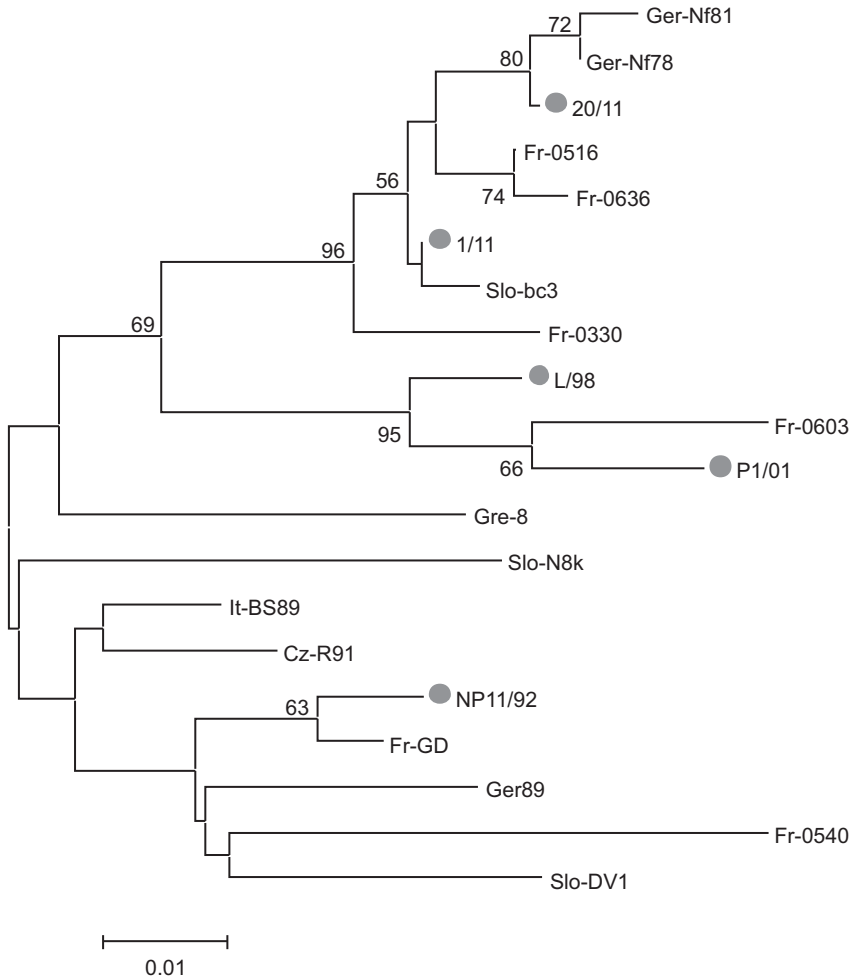


Fig. 1. Phylogenetic tree of 220 nucleotide gene fragment using neighbor-joining method. Bootstrap values greater than 50 % (for 1000 replicates) are given. The nucleotide sequences of Polish strains, marked by grey dots, are as follow: NP11/92, L/98, P1/01 (1), 1/11, 20/11; strain 4/11 is represented by 1/11. The sequences of 15 foreign strains used to construct phylogenetic tree are available in GenBank and originated from: Czech Republic, Cz-R91 (U65362); France, Fr – GD (Z69620), 0330 (AM887765), 0516 (AM933650), 0540 (AM887765), 0603 (AM933648), 0636 (AM933649); Germany, Ger – 89 (U09199), Nf81 (JN398482), Nf78 (JN398481); Italy, It-BS89 (X98002); Slovakia, Slo – DV1 (DQ862478), N8k (DQ862480), bc3 (DQ862479) and Greece – Gre-8 (AM048854).

the RT-PCR and purification of the amplified PCR products were carried out using commercial Qiagen kits (QIAamp® Viral RNA Mini Kit, OneStep® RT-PCR Kit and Gel Extraction Kit) according to the manufacturer's instructions. Primer sets used in RT-PCR (PS1 and P7) and nested-PCR (PS3, PS3', PS4 and PS4') were published earlier (Chrobocińska 2007). Nested-PCR products were sequenced in both directions on ABI Prism 377DNA Sequencer (Sequencing service, Institute of Biochemistry and Biophysics, Polish Academy of Sciences).

Genetic analysis was conducted on the basis of sequences derived from Polish EBHSV strains and other European virus sequences available in GenBank. The multiple sequence alignment of 220 nucleotides from the VP60 gene fragment (position 6464-6685) was used to generate a neighbor-joining tree using MEGA 5.2.1. software.

## Results and Discussion

The EBHSV was present in 6 of the total of 12 sampled hares; however, the nucleotide sequences were obtained for only 5 samples. They originated from diverse geographical areas of Poland (provinces of Lubuskie, Lubelskie and Świętokrzyskie). In the analysed genomic region of EBHSV, 4 strains (1/11, 4/11, 12/11 and 15/11) revealed 100% homology to each other. They represented one genotype of EBHSV, although they were collected from 2 farms located in different provinces (Lubuskie and Lubel-

skie). Despite the fact that the 1/11 strain showed identical sequence homology to 3 other Polish strains, it was classified as a new strain, due to small genomic variation when the fragment of 1005 nucleotides was studied (position 6009-7013, data not shown). Although virus strains 1/11 and 4/11 turned out to be various, they were identified on the same farm (Lubuskie Province). A similar case of two strains in one EBHS outbreak has already been described (Drews et al. 2011).

In the phylogenetic analysis presented in this paper, only new EBHSV strains (1/11, 4/11 and 20/11) with different nucleotide sequences were included. They were grouped together with foreign strains detected in recent years: French (0330, 0516, 0636), Slovakian (bc3) and German (Nf78, Nf81) (Fig. 1). Their close genetic relationship was confirmed by a high bootstrap value (96%). The analysis of the predicted 73 amino acid sequence of new Polish and European strains revealed the same substitutions of leucine by methionine (413 L→M,) and valine by threonine (423 V→T), caused by non-synonymous mutations C→A and GT→AC, respectively (data not shown). The substitution in position 413 was also demonstrated in 1 strain from Greece (Gre-8) and in position 423 in two strains from Poland (L/98, P1/01).

In this study, we described EBHSV strains currently existing in Poland and demonstrated their phylogenetic relationship with other European strains. Although Frolich *et al*. indicated that there is no clear relationship between strains, time of isolation and geographic origin (Frolich et al. 2007), the importance of geographic and temporal distributions of the virus strains in evolution has already been considered (Le Gall-Recule at al. 2006). The results suggested that diversity of EBHSV strains is probably connected with the time of their collection.

## References

Chrobocińska M (**2007**) Charakterystyka fenotypowa i molekularna krajowych szczepów wirusów krwotocznej choroby zajęcy (EBHSV) i krwotocznej choroby królików (RHDV). Habilitation dissertation, The National Veterinary Research Institute, Puławy, Poland.

Drews B, Szentiks CA, Roellig K, Fickel J, Schroeder K, Duff JP, Lavazza A, Hildebrandt TB, Goeritz F (**2011**) Epidemiology, control and management of an EBHS outbreak in captive hares. Vet Microbiol 154: 37-48.

Frolich K, Fickel J, Ludwig A, Lieckfeldt D, Streich WJ, Jurcik R, Slamecka J, Wibbelt G. (**2007**) New variants of European brown hare syndrome virus strains in free-ranging European brown hares (*Lepus europeus*) from Slovakia. J Wildl Dis 43: 89-96.

Le Gall-Recule G, Zwingelstein F, Laurent S, Portejoie Y, Rasschaert D (**2006**) Molecular epidemiology of European brown hare syndrome virus in France between 1989 and 2003. Arch Virol 151: 1713-1721.