Available online at www.sciencedirect.com
ScienceDirect
Veterinary Microbiology 127 (2008) 392–398
veterinary microbiology
www.elsevier.com/locate/vetmic

Short communication

Molecular epizootiology of equine arteritis virus isolates from Poland

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Received 10 April 2007; received in revised form 28 August 2007; accepted 11 September 2007

Abstract

Phylogenetic analysis was performed on the sequences of 44 Polish isolates of equine arteritis virus that were isolated from the semen of stallions from national and private studs, collected during 2001–2005. These sequences were also compared with 41 reference strains previously described and commonly used in phylogenesis. On the basis of the nucleotide sequence analysis of the ORF5 gene, encoding the glycoprotein GP5, it was demonstrated that the Polish EAV isolates belonged to two subgroups and showed the closest relationship to the European strains. Similar results were obtained using the nucleotide sequences of the ORF7 gene. The nucleotide identity between the ORF5 and ORF7 sequences of all Polish isolates was in the range of 80.1–99.0% and 93.6–100%, respectively. The analysis of genetic diversity within the ORF5 sequences enabled a retrospective epizootic investigation. This study suggested that some of the EAV shedding stallions were probably infected before they were moved to Poland.

Keywords: Equine arteritis virus; Phylogenetic analysis; Stallion

1. Introduction

Equine arteritis virus (EAV) is present in many horse populations throughout the world. The course of an EAV infection is usually subclinical. 30–60% of stallions become persistent carriers and can shed EAV within their semen for several weeks, months or even years. Mating of susceptible mares with EAV shedding stallions can result in embryo resorptions, abortions, infertility and sometimes death of the newborn foals, which leads to large economic losses in horse breeding. Persistently infected stallions are considered as the main reservoir of the virus; hence, the control of the disease should be based on the identification of these infected animals and the limitation of their use to breed previously infected or vaccinated mares only. Risk of EAV transmission from persistently infected stallions to other horses in the same holding by other that venereal routes should be also considered (Balasuriya et al., 1999, 2004; Guthrie et al., 2003).

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doi:10.1016/j.vetmic.2007.09.006

Although most EAV strains are of low virulence, outbreaks of equine viral arteritis with severe clinical symptoms still occur (Larsen et al., 2001; Szeredi et al., 2005). The studies showed that over 30% of abortions in pregnant mares in Poland could have been caused by EAV infections and the virus was a more frequent cause of abortions than equine herpes virus 1 (Golnik and Sordyl, 2004). The first outbreak of equine viral arteritis (EVA) in Poland was described at a thoroughbred stud in 1976–1977. The strain isolated at the outbreak was named Wroclaw-2 (Golnik and Michalak, 1978). In the following years, the presence of EAV specific antibodies was detected in 24% of stallions tested at the national reproduction centers in Poland (Golnik, 2000). Recent serological investigations among Polish stallions showed a decrease in the percentage of EAV infection to 17.4%. The probable cause of this drop was the efficient implementation of the regulations enforcing the requirement to test the stallions for EAV at the national reproduction centers in Poland at the beginning of 2001.

EAV belongs to the family Arteriviridae of the order Nidovirales (Cavanagh, 1997). The viral genome is composed of a single-stranded positive sense RNA enclosed in an icosahedral nucleocapsid and surrounded by a proteolipid envelope. To date, nine open reading frames (ORFs) in the EAV genome have been described which encode the viral replicase (ORFs 1a and 1b), two non-structural proteins GP3 and GP4 (ORFs 3 and 4) and five structural proteins E, GP2b (formerly G_S), GP5 (formerly G_L), M and N encoded by ORFs 2a, 2b, 5, 6 and 7, respectively (Snijder et al., 1999; Snijder and Meulenberg, 1998). Although only one serotype of EAV has been identified, field isolates can differ in their virulence, pathogenicity and antigenic properties. ORF5, encoding *N*-glycosylated protein GP5, is one of the most variable genes in the EAV genome. The GP5 ectodomain is the main antigen recognized by neutralizing antibodies (Chirnside et al., 1995). Specific reactions of neutralizing antibodies to viral antigen are up regulated by binding into four main sites (A–D) of GP5 glycoprotein (Balasuriya et al., 1997). Comparative analysis of the deduced GP5 amino acid sequence of EAV strains revealed three distinct variable regions V1–V3 (Balasuriya et al., 1995b). Genetic diversity of the ORF5 sequence is commonly used in phylogenetic analyses of EAV as well as some other Arteriviridae like porcine respiratory and reproductive syndrome virus (Balasuriya et al., 1995a; Stadejek et al., 1999, 2002; Hornyak et al., 2005; Larsen et al., 2001).

The data presented in this study is the first attempt to characterise the genetic diversity of EAV isolates present in horses in Poland using the analysis of ORF5 and ORF7 sequences from 44 EAV strains isolated from persistently infected stallions present at 18 different horse studs. Additionally, the analysis of phylogenetic relationship as a tool for tracing back the source of EAV infection was demonstrated.

2. Materials and methods

Forty-four semen samples, which proved positive for EAV isolation in RK 13 cell culture (ATCC CCL-37) and in diagnostic RT-PCR assays for EAV, collected between 2000 and 2005, were used in this survey. The material was obtained from stallions originating from small, private stables, national horse studs (over 200 horses in holding) and stallion depots from 11 (out of 16) regions of Poland (Table 1).

Total RNA was isolated directly from the semen samples of stallions using TRI Reagent (Sigma) according to the manufacturer's instruction. The samples were archived at −70 °C following RT-PCR. Two pairs of oligonucleotides were used to obtain sequences of ORF7 and ORF5. The primers OEVA14a and OEVA15, flanking a 395 bp fragment from within the nucleocapsid gene (ORF7) of EAV, have been described previously (Belak et al., 1994). The primers MLEAV1 (5′TCTTTTACGACTGGTACGTTGG3′) and MLEAV2 (5′AAAATCCCGTCACCACAAAA3′) AAATCCCGTCACCACAAAA3′) were designed using the reference sequence of EAV reference strain Bucyrus (Accession number DQ846750, starting at nt 11116 for the forward primer and nt 11920 for the reverse primer) with the program OLIGO v. 6.71 (Molecular Biology Insights, Inc., USA) and then used to amplify fragment of 822 bp corresponding to a region of ORF5. For further sequence comparison, a shorter region of 519 bp (nt 11296–11814 of the EAV strain Bucyrus genome), as used for of most of reference ORF5 sequences, was compared.

The RT-PCR assays were carried out using the one-tube Access RT-PCR System kit (Promega) using the