Veterinary Microbiology 172 (2014) 63–71

Contents lists available at ScienceDirect

Veterinary Microbiology

journal homepage: www.elsevier.com/locate/vetmic

Analysis of the genetic diversity of ovine herpesvirus 2 in samples from livestock with malignant catarrhal fever

George C. Russell [a,*], Sandra F. Scholes [b], David F. Twomey [c], Ann E. Courtenay [d], Dawn M. Grant [a], Bruce Lamond [a], David Norris [a,1], Kim Willoughby [e], David M. Haig [f], James P. Stewart [g]

[a] Moredun Research Institute, Pentlands Science Park, Penicuik EH26 0PZ, UK
[b] Animal Health and Veterinary Laboratories Agency (AHVLA), Pentlands Science Park, Midlothian, UK
[c] AHVLA Starcross Regional Laboratory, Staplake Mount, Starcross, Devon EX6 8PE, UK
[d] AHVLA Surveillance Centre, Division of Livestock Health and Welfare, School of Veterinary Science, Leahurst Campus, University of Liverpool, Wirral CH64 7TE, UK
[e] Moredun Scientific, Pentlands Science Park, Penicuik EH26 0PZ, UK
[f] School of Veterinary Medicine and Science, University of Nottingham, Sutton Bonington, Leicestershire LE12 5RD, UK
[g] Department of Infection Biology, Institute of Infection and Global Health, University of Liverpool, Liverpool Science Park IC2, L3 5RF, UK

ARTICLE INFO

Article history:
Received 7 February 2014
Received in revised form 4 April 2014
Accepted 15 April 2014

Keywords:
Inflammatory disease
Malignant catarrhal fever
Sequence analysis
Multi-locus Sequence typing
Polymorphism

ABSTRACT

In order to define better virus isolates from animals with malignant catarrhal fever (MCF), segments of three genes of ovine herpesvirus-2 were amplified from diagnostic samples representing MCF cases with a range of clinical presentations in cattle, including head and eye, alimentary and neurological. The variation within each gene segment was estimated by DNA sequencing, which confirmed that the newly-annotated Ov9.5 gene was significantly more polymorphic than either of the other loci tested (segments of ORF50 and ORF75), with alleles that differed at over 60% of nucleotide positions. Despite this, the nine Ov9.5 alleles characterised had identical predicted splicing patterns and could be translated into Ov9.5 polypeptides with at least 49% amino acid identity. This multi-locus approach has potential for use in epidemiological studies and in charactering chains of infection. However there was no association between specific variants of OvHV-2 and the clinical/pathological presentation of MCF in the cattle analysed.

1. Introduction

Gammaherpesviruses (γHV) can replicate in and latently infect lymphoid cell types and are associated with lymphoproliferative diseases and tumours (Ackermann, 2006; Barton et al., 2011; Nash et al., 2001). They share similarities of size, sequence and genome organisation. Evolution within the subfamily *Gammaherpesvirinae* is reflected in the colinearity of most γHV genome sequences, with approximately 50 genes considered to be conserved across the group of fully-sequenced γHV (McGeoch et al., 2005). The genes of most γHV are named with respect to the genome of the prototype virus of this family, herpesvirus saimiri (HVS), with 75 open reading frames (ORF) numbered from the left of the genome (Albrecht et al., 1992). These genes encode the components of the virus capsid, tegument and envelope; proteins involved in replication of the virus genome; regulatory proteins that control the lytic cycle and latency; and

* Corresponding author. Tel.: +44 131 445 6149; fax: +44 131 445 6111.
E-mail addresses: george.russell@moredun.ac.uk, george.russell@mri.sari.ac.uk (G.C. Russell).
[1] Present address: Scottish Lowlands Forest District, Five Sisters House, Five Sisters Business Park, West Calder EH55 8PN, UK.

http://dx.doi.org/10.1016/j.vetmic.2014.04.011
0378-1135/

proteins that manipulate the metabolism of the host to benefit viral replication and persistence. Comparison of HVS with other γHV shows that each virus carries a complement of virus genes that do not have homologues in the HVS genome (McGeoch, 2001). These genes are termed unique genes, although they may be shared within groups of related viruses. They are annotated by a prefix letter specific to the virus and are numbered from the left of the genome. Unique genes in γHV have been identified by bioinformatic analysis, comparative genomics and by analysis of cDNA or protein sequences (Albrecht et al., 1992; Coulter et al., 2001; Hart et al., 2007; Hughes et al., 2010; Mills et al., 2003; Russell et al., 2013).

Several ruminant gammaherpesviruses of the *Macavirus* genus (Davison et al., 2009) are associated with the lymphoproliferative disease malignant catarrhal fever (MCF). The best studied of these are ovine herpesvirus 2 (OvHV-2) and alcelaphine herpesvirus 1 (AlHV-1). MCF occurs when virus shed in the mucous secretions of reservoir host species (sheep for OvHV-2 and wildebeest for AlHV-1), which are infected efficiently and without obvious clinical signs, infects susceptible species such as cattle, bison, deer and pigs (Russell et al., 2009). MCF is an often-fatal systemic disease that is generally sporadic, affecting individual animals within a group, but can occasionally cause losses of up to 40% of a herd (Russell et al., 2009; World Organisation for Animal Health (OIE), 2008). The clinical signs of MCF can be varied and several distinct disease presentations have been described, including peracute, head and eye, alimentary and neurological (World Organisation for Animal Health (OIE), 2008). Most MCF cases in cattle present with fever, depression and lymphadenopathy; while the common head and eye form is further characterised by nasal and ocular secretions, corneal opacity, skin lesions and multifocal necrotic lesions of the gums, tongue and palate (Otter et al., 2002). In the peracute form, sudden death may occur, though depression followed by diarrhoea, with death occurring within a few days has also been reported. In the alimentary form, haemorrhagic diarrhoea may also be found (Holliman et al., 2007), while nervous signs, ataxia, and blindness have been reported in the neurological form (Mitchell and Scholes, 2009). Although MCF is generally considered a disease with a case fatality rate approaching 100%, reports of recovery from clinical MCF and chronic infection have also been published (Milne and Reid, 1990; OToole et al., 1997; Penny, 1998; Twomey et al., 2002).

The genomes of AlHV-1 (Ensser et al., 1997) and OvHV-2 (Hart et al., 2007; Taus et al., 2007; Jayawardane et al., 2008) have been fully sequenced, demonstrating conservation of γHV genome structure and possession of a similar complement of unique genes (Russell et al., 2009). Recently, analysis of virus gene expression in AlHV-1 infected cells revealed the presence of a novel spliced gene (Russell et al., 2013) that encoded a secreted glycoprotein. This gene, termed A9.5, had not been previously predicted because of the small size of the coding exons and because no similar protein was present on any database. Predicted segments of protein sequence with similarity to these exons were found at the same position in OvHV-2, such that the homologous gene Ov9.5 could be identified despite having only 33% translated amino acid identity to the A9.5 polypeptide. Notably, the two published OvHV-2 genome sequences contained distinct Ov9.5 genes, which had equivalent positions and predicted splicing patterns but shared only 60% nucleotide identity (Russell et al., 2013). The low degree of identity between the two alleles of Ov9.5 suggests distinct histories, selective regimes or functions (Russell et al., 2013). It may therefore be relevant that one Ov9.5 sequence was from a clinical case of MCF in a British cow while the other was obtained from OvHV-2 virions from sheep nasal secretions in the USA (Hart et al., 2007; Taus et al., 2007).

In order to compare sequence variation within the Ov9.5 gene with other loci in OvHV-2, two additional genes were selected for comparative sequence analysis. These were: a segment of the ORF75 gene, encoding the virion enzyme formylglycineamide ribotide amidotransferase (FGARAT), routinely used as the target of a diagnostic PCR assay for OvHV-2 (Baxter et al., 1993); and a segment of the ORF50 gene, encoding RTA, a transcription factor involved in lytic cyle activation, selected because of its important role in virus regulation. The ORF73 (latency-associated nuclear antigen) locus was also considered as a target for PCR but initial studies showed that this gene could not be reliably amplified from clinical case material despite the use of published primer sets (Coulter and Reid, 2002) or newly designed nested primers (GC Russell, unpublished data). This is likely to be a consequence of the size and repetitive nature of this gene combined with the relatively low viral load in the samples used for analysis.

In this paper we analyse genetic variation in OvHV-2 from clinical case samples by looking at three loci to address the hypothesis that the highly polymorphic Ov9.5 gene is a useful epidemiological marker of OvHV-2 strain variation. We also examine the possibility that distinct strains of OvHV-2 may be responsible for different presentations of MCF in cattle.

2. Materials and methods

2.1. Clinical samples

All DNA samples used in this work were extracted from material submitted to Moredun Research Institute for PCR-based testing in support of a diagnosis of MCF. Prior to 2006, DNA samples were purified from peripheral blood mononuclear cells (PBMC) or from tissues from MCF-suspect cases by a standard phenol-based method (Sambrook et al., 1989), while samples collected since 2006 were purified by a column-based method that did not use organic solvents (DNeasy mini, Qiagen, Crawley, UK).

OvHV-2 positive samples were selected according to reported clinical signs, representing a range of clinical disease presentations as documented in Table 1 and summarised as follows: alimentary, 11 cases, including herds A and E from the report by Holliman et al. (2007); head & eye, four recovered cases described by Twomey et al. (2002); neurological, two cases reported by Mitchell and Scholes (2009). In addition, seven DNA samples from two MCF outbreaks (head and eye form) in different parts of the UK (including one sample from an in-contact sheep) and five samples from sporadic MCF cases (where no other