Recombination in alphaherpesviruses was first described more than sixty years ago. Since then, different techniques have been used to detect recombination in natural (field) and experimental settings. Over the last ten years, next-generation sequencing (NGS) technologies and bioinformatic analyses have greatly increased the accuracy of recombination detection, particularly in field settings, thus contributing greatly to the study of natural alphaherpesvirus recombination in both human and veterinary medicine. Such studies have highlighted the important role that natural recombination plays in the evolution of many alphaherpesviruses. These studies have also shown that recombination can be a safety concern for attenuated alphaherpesvirus vaccines, particularly in veterinary medicine where such vaccines are used extensively, but also potentially in human medicine where attenuated varicella zoster virus vaccines are in use. This review focuses on the contributions that NGS and sequence analysis have made over the last ten years to our understanding of recombination in mammalian and avian alphaherpesviruses, with particular focus on attenuated live vaccine use.
Highlights:

- Natural (field) recombination in alphaherpesviruses is linked with live attenuated vaccine usage.

- Natural recombination drives evolution and pathogenicity of alphaherpesviruses.

- Advances in next-generation sequencing techniques have helped to provide accurate data in regards to natural (field) recombination.
Natural recombination in alphaherpesviruses: insights into viral evolution through full genome sequencing and sequence analysis

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Abstract:
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Keywords: Alphaherpesvirus, natural recombination, next-generation sequencing, attenuated live vaccine.
Contents:

1. Introduction ................................................................. 4

2. Natural recombination in human alphaherpesviruses ....................... 6
   2.1 Herpes simplex virus-1 ............................................. 6
   2.2 Herpes simplex virus-2 ............................................. 8
   2.3 Varicella zoster virus ............................................. 9

3. Natural recombination in other mammalian herpesviruses ................... 12
   3.1 Equine alphaherpesviruses ....................................... 13
   3.2 Pseudorabies virus .............................................. 14
   3.3 Feline herpesvirus-1 ............................................. 15

4. Natural recombination in avian alphaherpesviruses ........................... 16
   4.1 Marek’s disease virus ........................................... 16
   4.2 Infectious laryngotracheitis virus ................................ 17

5. Conclusions ...................................................................... 18

Acknowledgements .................................................................. 20

Conflict of interest .................................................................. 20

References ............................................................................. 20
1. Introduction

Viruses belonging to the order *Herpesvirales* have a double stranded DNA genome and have been isolated from a wide variety of host including mammals, birds, reptiles, fish and invertebrates (Davison, 2010). Three large subfamilies arose within the family *Herpesviridae* over 80 million years ago. One of these, the subfamily *Alphaherpesvirinae*, includes avian and mammalian alphaherpesvirus lineages (Davison, 2010; McGeoch et al., 1995). The evolution of many viruses in this subfamily has been attributed, in part, to the process of recombination. The contribution that recombination makes to the evolution and diversity of alphaherpesviruses is of particular importance as these viruses have a DNA polymerase with a highly efficient proof-reading activity and exonuclease activity (Javier et al., 1986; Lee et al., 2012; Thiry et al., 2005), resulting in low point genetic mutation rates. In herpes simplex virus-1 (HSV-1) the mutation rate is as low as 0.026 to 0.0027 (Drake and Hwang, 2005).

Recombination is the process in which new genetic material (offspring) is generated by shuffling two different DNA sequences from viruses infecting the same host cell at the same time. High rates of *in vivo* intra-species homologous recombination have been demonstrated after experimental co-inoculation of different strains of HSV-1 into mice (Kintner et al., 1995), bovine herpesvirus 1 (BoHV-1) into calves (Schynts et al., 2003), and pseudorabies virus (PRV) into sheep and pigs (Christensen and Lomniczi, 1993; Henderson et al., 1990). *In vitro* intra-species co-inoculation experiments have demonstrated recombination in different alphaherpesviruses strains into cell cultures, including HSV-1 (Kintner et al., 1995), BoHV-1 (Muylkens et al., 2009), varicella zoster virus (VZV) (Dohner et al., 1988), feline herpesvirus 1 (FeHV-1) (Fujita et al., 1998) and PRV (Henderson et al., 1990). *In vitro* recombination has been detected at a lower rate than in *in vivo* experiments, possibly due to the reduced number of viral replication cycles possible in cell cultures. Therefore, factors that influences the number of viral replication cycles, such as latency/reactivation and use of vaccines that replicate after vaccination, should be considered as factors that may increase the likelihood of recombination. Recombination occurs most commonly between different
strains of the same virus species (intra-species recombination) but inter-species recombination is also possible and has been detected in experimental studies between caprine herpesvirus-1 and -2, and also between BoHV-1 and bovine herpesvirus-5 (BoHV-5) (Meurens et al., 2004). In field samples inter-species recombination has been detected between equine herpesviruses 1 and 4 (EHV-1 and EHV-4, respectively) (Pagamjav et al., 2005).

Many biological features of alphaherpesviruses, including their infection of epithelial surfaces, rapid infectious cycle, establishment of latent infection with periodic reactivation and high prevalence of infection in many host populations, create a favourable environment for co-infection of host cells, and hence for recombination. The viral, host and cell conditions that influence the likelihood of recombination in vivo and in vitro under laboratory conditions have been reviewed previously (Thiry et al., 2005). The molecular basis of alphaherpesvirus recombination has also been recently reviewed and is hypothesized to be similar to that described for lambda bacteriophages (Lo Piano et al., 2011; Weller and Sawitzke, 2014).

Early studies of alphaherpesvirus recombination used strain virulence as a marker to detect recombinants (Wildy, 1955). Analysis of partial genome sequences were then used extensively to study recombination in several alphaherpesviruses, using tools such as PCR followed by restriction endonuclease cleavage fragment analysis of PCR products (PCR plus restriction fragment length polymorphism [PCR-RFLP]), gene deletion mutants, PCR hydrolysis probe assays and bioinformatic comparisons of partial genome sequences to detect recombination (Bowden et al., 2004; Christensen and Lomniczi, 1993; Dangler et al., 1993; Dohner et al., 1988; Glazenburg et al., 1994; Henderson et al., 1990; Javier et al., 1986; Kintner et al., 1995; Muylkens et al., 2009; Norberg et al., 2004; Sakaoka et al., 1995; Sakaoka et al., 1994; Schyns et al., 2003; Umene and Sakaoka, 1997). More recently, lower costs, improved technologies and greater access to next generation sequencing (NGS) techniques (Capobianchi et al., 2013; Pareek et al., 2011), statistical analysis (Bruen et al., 2006; Posada, 2002) and software to detect and estimate the likelihood of recombination (Huson and Bryant, 2006; Kosakovsky Pond et al., 2006; Kuhner, 2006; Lole et al.,
1999; Martin et al., 2010; Martin et al., 2011; Martin et al., 2015; Pond and Frost, 2005; Wilson and McVean, 2006) have helped us to better understand recombination, and thereby provide insights into the role of recombination in the natural evolution of alphaherpesviruses (Burrel et al., 2015; Hughes and Rivailler, 2007; Kolb et al., 2013; Kolb et al., 2015; Lamers et al., 2015; Lee et al., 2013; Lee et al., 2012; Newman et al., 2015; Norberg et al., 2015; Norberg et al., 2007; Norberg et al., 2006; Norberg et al., 2011; Peters et al., 2006; Szpara et al., 2014; Vaz et al., 2016a; Vaz et al., 2016b; Ye et al., 2016; Zhao et al., 2015). This review aims to summarise and update our understanding of natural recombination in alphaherpesviruses and the influence of natural recombination on viral evolution, focusing on the contributions that full genome sequencing and sequence analysis have made to this field over the last 10 years. This review covers natural recombination in human alphaherpesviruses, other mammalian alphaherpesviruses and avian alphaherpesviruses.

2. Natural recombination in human alphaherpesviruses

Herpes simplex virus-1, -2 and VZV are important causes of human disease worldwide. Infection with HSV-1 is commonly associated with ulcerated oral lesions and HSV-2 with genital lesions, although both viruses can cause lesions at both anatomical sites (Lowhagen et al., 2002). Additionally, HSV-1 can cause keratitis and subsequent blindness, along with sporadic encephalitis (Liesegang, 2001). Infection with VZV causes varicella (chickenpox) and herpes zoster (shingles) (Zerboni et al., 2014). Evidence of natural recombination has been available since 2004 for HSV-1, 2003 for VZV, and 2007 for HSV-2. These studies have demonstrated that these three human alphaherpesviruses show differences in recombination (Table 1).

2.1 Herpes simplex virus-1

Early HSV-1 studies in mice demonstrated that a high proportion of recombinant viruses were generated following co-inoculation, and many of the recombinants had higher levels of virulence...
than the parental strains (Brandt and Grau, 1990; Kintner et al., 1995). This potential for recombination to result in increased virulence highlighted the importance of studying HSV-1 recombination in clinical settings. In 2004, Bowden et al., sequenced approximately 4% of the HSV-1 genome in 14 clinical samples from the UK and Korea, performed phylogenetic network analyses on the data to estimate recombination, and concluded that recombination plays a major role in generating diversity within HSV-1 (Bowden et al., 2004). In the same year, Norberg et al., sequenced approximately 2.3% of the HSV-1 genome in 28 clinical samples from Sweden, also detecting recombination (Norberg et al., 2004).

From 2011 onwards, techniques shifted to detection of natural HSV-1 recombination by bioinformatic analysis of whole genome sequences obtained by NGS, providing a higher level of accuracy in identifying and characterising these phenomena (Kolb et al., 2011; Kolb et al., 2013; Norberg et al., 2011; Szpara et al., 2014). In 2011 the genomes of ten clinical and two laboratory HSV-1 strains isolated in 1972, were sequenced and analysed. Significant recombination was detected, including recombination events between the HSV-1 field strains F and 17. Additionally, the full genome sequences of these 12 HSV-1s facilitated classification of HSV-1 into 3 clades: A, B and C (Norberg et al., 2011). Kolb et al reached similar conclusions about the number of HSV-1 clades (Kolb et al., 2011). In regard to recombination, Kolb et al., (2011) showed that some field strains (TFT401 and CJ970) were unstable in their phylogenetic classification using the whole genome sequence and identified cross over points using similarity plots in the UL1, UL11, UL43, UL49A, US4 and US7 genes. Additionally, they found that some nucleotide sequences that code for selected proteins resulted in variable phylogenetic groupings depending on the parameters used to build the phylogenetic trees. They concluded that recombination was likely and that each viral genome is a unique mosaic (Kolb et al., 2011).

The most recent and comprehensive studies of HSV-1 genetic diversity, recombination and genome evolution are those that have examined full genome sequences of HSV-1 from four continents. Kolb
154 *et al.*, (2013) examined 31 full genome sequences as well as partial genome sequences obtained from the NCBI reference database. Szpara *et al.*, (2014) examined whole genome sequences obtained by NGS of 20 field strains of HSV-1 obtained from China, Japan, Kenya and South Korea and compared them with the genome sequences available for HSV-1 strains from the United States (US), Europe and Japan. Both these studies confirmed that recombination in HSV-1 is widespread, frequent, historical and ongoing (Kolb *et al.*, 2013; Szpara *et al.*, 2014). In addition, these two studies found that the HSV-1 strains clustered into six groups, rather than the three groups described previously. The clustering correlated with the geographic origin of the isolates, highlighting the need to include isolates from different regions in order to comprehensively examine phylogeny and recombination (Kolb *et al.*, 2013; Szpara *et al.*, 2014).

164 The high levels of HSV-1 recombination detected in these studies have been hypothesised to be due to the co-existence of many different HSV-1 strains within the same geographical region (Norberg *et al.*, 2004; Schmidt-Chanasit *et al.*, 2009) and to the high number of HSV-1 replication cycles that may arise from more frequent HSV-1 reactivation from latency, compared to HSV-2 or VZV (Kaufman *et al.*, 2005; Wang *et al.*, 2010). This suggests that the latency and reactivation characteristics of alphaherpesviruses are also factors that can influence recombination, with viruses that establish and re-activate from latency more frequently having a higher likelihood of recombination.

173 2.2 Herpes simplex virus-2

174 The first study to detect recombination in HSV-2 using bioinformatics analyses of partial DNA sequences from clinical isolates was published by Norberg *et al.*, (2007). Approximately 3.5% of the genome of 47 clinical isolates of HSV-2 from Norway, Sweden, and Tanzania was examined and it was found that HSV-2 was clustered into 2 genogroups, rather than the 3 groups seen in HSV-1 isolates using similar techniques. Isolates from Norway and Sweden were clustered into only one genogroup. It was hypothesised that the lower level of genetic diversity seen in HSV-2,
compared to HSV-1, was directly related to recombination (Norberg et al., 2007). Importantly, however, these conclusions were based on the analysis of only a limited number of samples, and of partial genome sequences. Only three genes within the unique short (US) region of the HSV-2 genome were examined (US4, US7 and US8) (Norberg et al., 2007). Studies on HSV-1 had detected variation in diversity across the whole genome, which can influence recombination analysis (Szpara et al., 2014). Thus, in order to comprehensively examine recombination in HSV-2, and also compare recombination between HSV-1 and HSV-2, an analysis of HSV-2 recombination analyses at the whole genome level was required.

Prior to 2014 there were only two full genome sequences for HSV-2, one published in 1998 (Dolan et al., 1998), and another one in 2014 (Colgrove et al., 2014). Kolb et al., (2015) determined the complete genome sequences of an additional six HSV-2 clinical isolates. Bootscan analysis of the eight complete genome sequences demonstrated that the HSV-2 genomes were mosaics, suggesting frequent recombination randomly along the genome (Kolb et al., 2015). In a separate study, 34 near complete genome sequences were determined for HSV-2 isolates from Africa, USA and Japan. Bootscan and phylogenetic analyses of these sequences suggested that HSV-2 had five major crossover points and that recombination in HSV-2 did not occur as frequently as in HSV-1 (Newman et al., 2015). Another recent study has reported that a new HSV-2 variant (HSV-2v) from west and central Africa (mostly from immunocompromised patients infected with human immunodeficiency virus) differed significantly from the classical HSV-2 prototype and contained a UL30 gene (encoding DNA polymerase) that clustered closely with the chimpanzee herpesvirus (ChHV), providing evidence of an inter-species recombination event (Burrel et al., 2015). Herpes simplex virus 2 and ChHV have genetically similar genomes with 88.3% pairwise identity and 88.3% identical sites between ChHV (Genbank accession number JQ360576) and HSV-2, (Genbank accession number Z86099) as determined using Multiple Alignment with Fast Fourier Transformation (MAFFT) version 7 within Geneious V8.0.4 (Katoh and Standley, 2013). A high level of identity between viruses has been shown to play a role in promoting recombination. This
level of identity is similar to what has been described for EHV-1 and -4, another pair of alphaherpesviruses in which natural inter-species recombination has been detected (Pagamjav et al., 2005).

2.3 Varicella zoster virus

Varicella zoster virus is the only human alphaherpesvirus for which live attenuated vaccines are in widespread use. These live attenuated vaccines (Takahashi et al., 1974) contain a heterogeneous mixture of related VZV haplotypes (Depledge et al., 2014) and are used in several countries including Japan, Korea, the US, Canada, Australia, Germany, Costa Rica, Uruguay, and Qatar (Norberg et al., 2015). As VZV recombination has been observed in cell culture (Dohner et al., 1988) there is potential for natural recombination, including between vaccine and wild type viruses, to occur (Quinlivan et al., 2009). This has been the focus of a number of studies examining VZV recombination. VZV recombination has been investigated using partial and full genome sequence analyses to identify viral groups (clades), and to detect recombination events (Norberg et al., 2015; Norberg et al., 2006; Norberg et al., 2011; Peters et al., 2006).

Early studies used restriction endonuclease digestion profiles to examine differences between VZV genomes (Takada et al., 1995). Later, DNA sequencing and bioinformatic analyses were used to classify, determine similarities and detect recombination events between VZV isolates. Single nucleotide polymorphism (SNP) analysis (Wagenaar et al., 2003), heteroduplex mobility assays to locate informative SNPs along the genome (Barrett-Muir et al., 2003) and targeted sequencing of different regions of the VZV were used to detect and compare SNP patterns (Loparev et al., 2004).

These studies showed that recombination occurs among VZV isolates and also enabled the classification of VZV isolates into different phylogenetic groups. Loparev et al., (2004) were able to classify 326 VZV isolates from the six continents into the European (E), Japanese (J) and the Mosaic (M) groups by sequencing and analysing approximately 0.3% of the full VZV genome sequence. This study also sequenced and analysed approximately 6.9% of the full VZV genome sequence.
sequence of 16 isolates. Specifically, the M group was identified as carrying SNP patterns of both
the E and J group, and it was hypothesised that M strains resulted from recombination after mixed
infection (Loparev et al., 2004). A separate study classified isolates into four major clades, with
clade A containing European/North American (Dumas) isolates, clade B Japanese (vaccine-Oka)
isolates, clade C Asian-like isolates sharing some European/North American features, and clade D
containing European/North American-like isolates sharing some features of the Asian strains
(Wagenaar et al., 2003). These classifications provided the first insight into natural recombination
in VZV, but more detailed understanding was later achieved by full genome sequence analyses.
Complete genome sequence analyses were first used to detect natural VZV recombination in 2006
(Norberg et al., 2006; Peters et al., 2006). The two studies reporting these findings had different
foundations but both reached similar conclusions about recombination. One investigated
recombination within the clades C and D (Asian-like and European/North American-like,
respectively) at the full genome level (Peters et al., 2006). This study determined the full genome
sequences of 11 VZV isolates that were considered representative for Canada and the USA, and
compared them to seven other sequences that were publicly available (Peters et al., 2006). They
detected evidence of recombination within a Canadian isolate (VZV-8) within clade C that
contained mixtures of genetic characteristics from clade A (Dumas strain from Europe/North
America) and clade B (pOka strain from Japan). It was hypothesised that this isolate arose as a
result of a recombination event between the vaccine strain vOka from Japan and VZV field strains
from Canada, as the vaccine strain is used in Canada (Peters et al., 2006). However, as the vaccines
contains mixtures of distinct genetic subtypes (Quinlivan et al., 2005; Vassilev, 2005) and the
sequences available at the time in 2006 did not include all the strains within the vaccine, it was not
possible to reach this conclusion definitely (Peters et al., 2006).
The second study (Norberg et al., 2006) determined the full genome sequences of two VZV strains
(DR and 123) that were classified previously into group M (mosaic) by Loparev et al., (2004) and
thus contained genetic features from groups E (European) and J (Japanese). At the whole genome
level, the DR and 123 strains were shown to be well separated from each other and from groups E and J. Thus, group M was further divided into M1, represented by the 123 strain, and M2, represented by the DR strain. Bootscan analysis suggested that that the DR and 123 strains contain putative recombination dependent sites. Specifically, some genomic regions clustered with pOka, while other regions of the VZV genome clustered with the European strains (Norberg et al., 2006).

Subsequent phylogenetic network analysis revealed that the SVETA strain, which is a Russian isolate and was thought to belong to clade 1 (European clade), had been involved in an intra-clade recombination event (Norberg et al., 2011), and significant intra-clade recombination events were found among other VZV isolates (Zell et al., 2012).

After intra-clade recombination was observed, it was hypothesised that an increase in the number of full genome sequences available for analysis would reveal a greater number of recombinants. Additionally, it was hypothesised that human migration, along with widespread distribution of the attenuated VZV vaccine strains (Takahashi et al., 1974), may contribute to an apparent disappearance of VZV clades as recombination events would result in isolates becoming genomic mosaics similar to HSV-1 (Norberg et al., 2004; Sauerbrei and Wutzler, 2007; Sauerbrei et al., 2008). However, recent studies that have included several isolates from different continents have shown that the only clade to consistently display a relatively high level of recombination is the clade that contains the pOka and vOka vaccine strains (Norberg et al., 2015). Detection of recombination in the other clades appears to be dependent on the analytical method used to detect evidence of recombination (Norberg et al., 2015).

The relatively low rate of recombination in VZV compared to HSV-1 may be due to the distinct biology and epidemiology of VZV (Kaufman et al., 2005; Schmidt-Chanasit et al., 2009; Wang et al., 2010) as well as geographical separations of strains (Norberg et al., 2004; Schmidt-Chanasit et al., 2009). However, ongoing monitoring of recombination in field isolates of VZV is needed, as recombination between attenuated viruses such as vaccine strains has been detected, to create recombinant virulent progeny in other alphaherpesviruses (Lee et al., 2012), as well as in several
other virus families (Becher et al., 2001; Camus-Bouclainville et al., 2011; Chong et al., 2010; Cuervo et al., 2001; Dahourou et al., 2002; Holmes et al., 1999; Liu et al., 2003; Norberg et al., 2013; Seligman and Gould, 2004; Wenhui et al., 2012).

3. Natural recombination in other mammalian herpesviruses

Over the past ten years natural recombination has been assessed in alphaherpesviruses from five non-human mammalian hosts; EHV-1, EHV-4, EHV-9, FeHV-1 and PRV (Greenwood et al., 2012; Pagamjav et al., 2005; Vaz et al., 2016a; Vaz et al., 2016b; Ye et al., 2016) (Table 2). This represents only a small proportion of the alphaherpesviruses of importance in veterinary medicine. There have been, however, other efforts to better understand recombination in a wider range of mammalian alphaherpesviruses using experimental in vitro and in vivo studies, including BoHV-1, BoHV-5 (Meurens et al., 2004; Muylkens et al., 2009; Schynts et al., 2003), PRV (Christensen and Lomniczi, 1993; Henderson et al., 1990) and FeHV-1 (Fujita et al., 1998). Live attenuated vaccines are used extensively in veterinary medicine and there is clear evidence that attenuated live vaccines can naturally recombine to generate more virulent and dominant progeny (Lee et al., 2012). Therefore, the study of natural recombination in vaccinated and unvaccinated animals is of importance in improving animal health and disease control in the field of veterinary medicine.

3.1 Equine alphaherpesviruses

Equine herpesviruses 1 and 4 cause significant losses in horse industries worldwide (Allen et al., 2004). Equine herpesvirus-1 and 4 are genetically similar (Telford et al., 1998), but they differ significantly in terms of their pathogenesis and epidemiology (Allen et al., 2004; Patel and Heldens, 2005). Infection with EHV-1 causes respiratory disease in young horses, myeloencephalitis in older horses, abortion in mares and systemic perinatal disease (Allen et al., 2004; Patel and Heldens, 2005). Infection with EHV-4 also causes upper respiratory tract infection, but EHV-4 infection rarely induces systemic disease or abortions in mares, probably because of its limited capacity to
infect mononuclear cells, and therefore produce viraemia (Patel and Heldens, 2005; Vandekerckhove et al., 2011). The differences in the epidemiology of EHV-1 and EHV-4 have been well described (Allen et al., 2004; Patel and Heldens, 2005). One key epidemiological difference between these viruses is the substantially lower prevalence of infection with EHV-1 compared to that seen for EHV-4 (Gilkerson et al., 1999).

Natural recombination in EHV was first reported by Pagamjav et al., (2005). The field isolate EHV-1 B was shown to have arisen as a result of an inter-species recombination event between EHV-1 and EHV-4, and then spread among horse populations to become a dominant strain (Pagamjav et al., 2005). The recombination event described by Pagamjav et al., (2012) was the first evidence of natural inter-species recombination involving EHV-1. In 2012 another natural recombination event involving EHV-1 and equid herpesvirus 9 (EHV-9) was detected in a zoo in Germany by Greenwood et al., (2012) following analysis of the sequence of six virus genes using a distance based method within the Recombination Analysis Tool 1.0 (RAT v1.0). Interestingly, the recombinant was isolated from a polar bear with fatal encephalitis, even though neither EHV-1 nor EHV-9 naturally infect polar bears (Greenwood et al., 2012). More recent work has suggested that the recombination event involving these two viruses most likely occurred in zebras and was then transmitted to the polar bear (Abdelgawad et al., 2016). This recombination event has some similarities to the one detected between the HSV-2 and ChHV (Burrel et al., 2015), as both reports shown recombination within the UL30 gene (Burrel et al., 2015; Greenwood et al., 2012). The significance of the UL30 gene as a site of recombination, and potentially as an influence on the host range of the resultant recombinant viruses, warrants investigation in future studies.

The high level of genetic similarity between EHV-1 and EHV-9, and also between EHV-1 and EHV-4 (Telford et al., 1992; Telford et al., 1998) are likely to have facilitated these inter-species recombination events (Pagamjav et al., 2005). Alignment of EHV-1 genome sequences (Genbank accession numbers: AY464052, KF644566, KF644567, KF644568, KF644570, KF644572, KF644576, KF644577, KF644578, KF644579, KT324724, KT324725, KT324726, KT324727,
KT324728, KT324729, KT324730, KT324731, KT324732, KT324733, KT324734, NC_001491) and EHV-4 partial and full genome sequences (Genbank accession numbers: KT324735, KT324736, KT324737, KT324738, KT324739, KT324740, KT324741, KT324742, KT324743, KT324744, KT324745, KT324746, KT324747, KT324748, NC_001844) showed there was 86.6% pairwise identity and 70.6% identical sites between EHV-1 and EHV-4. Alignments between whole genome sequences of EHV-1 (Genbank accession numbers listed above) and EHV-9 (Genbank accession number: NC_011644) showed 98% pairwise identity and 87.9% identical sites. Both alignments were done by using MAFFT version 7 within Geneious V8.0.4 (Katoh and Standley, 2013).

Recently, our laboratory has determined the complete genomic sequences of 11 EHV-1 and 14 EHV-4 isolates from Australia and New Zealand (Vaz et al., 2016a). Phylogenetic analysis of EHV-4 isolates revealed evidence of widespread recombination. In contrast, analyses of the 11 EHV-1 isolates from Australia and New Zealand, along with another 13 international EHV-1 isolates, detected limited or no evidence of recombination, depending on the method of analysis used. Pathogenesis and epidemiology can influence recombination as both these factors have an impact on the ability of viral infections to overlap in space (i.e. the same cell) and time (Thiry et al., 2005). Other factors that promote alphaherpesviruses recombination include similar high loads of each co-infecting virus, similar levels of virulence and invasiveness, and similar tissue distributions of the co-infecting viruses (Thiry et al., 2005). EHV-1 and EHV-4 infections of the respiratory tract of horses result in similar viral titres, replication kinetics and durations of virus infection (Allen et al., 2004). A key difference between the pathogenesis of the two viruses lies in the ability of EHV-1 to efficiently penetrate the basement membrane of the respiratory mucosa (Vandekerckhove et al., 2011) and disseminate to other sites, including the vascular endothelium of the placenta and central nervous system, via a leukocyte-associated viraemia (Gryspeerdt et al., 2010). This involves additional episodes of viral amplification compared to EHV-4, which would seemingly increase the opportunities for recombination in EHV-1 if all other factors were constant. However, the much
higher frequency of natural recombination in EHV-4 compared to EHV-1 suggests that other factors, such as a lower prevalence of infection with EHV-1, may reduce opportunities for co-infection and may have a greater impact on natural recombination (Vaz et al., 2016a). It is interesting to note that, similar to the situation with HSV-1 and HSV-2, less recombination is evident in the less genetically diverse EHV-1, compared to the more genetically diverse EHV-4, but the relationship between the level of recombination and the level of genetic diversity requires further investigation.

3.2 Pseudorabies virus

Pseudorabies virus is the aetiological agent of Aujeszky’s disease in pigs and causes economic loss in the pig industry. Although PRV has been the target of eradication programs in some countries, it remains endemic in some regions including Asia, west/east Europe and South America. Pigs infected with PRV display a range of clinical signs, depending of the age of the affected animal, including neurological, respiratory and reproductive disease. Pseudorabies virus can also infect and cause disease in a wide variety of other hosts (Mettenleiter, 2008), including dogs, cats, cattle and small ruminants.

A live attenuated vaccine, Bartha-K61, has been used to control disease due to PRV infection in many countries, including China. Despite vaccination programs, disease outbreaks caused by new PRV variants have been reported in China since 2011 (Luo et al., 2014). The first study using whole genome sequence analysis to detect natural recombination in PRV has been published recently (Ye et al., 2016). This study has shown that a historical Chinese PRV strain (SC) isolated during the 1980s is a recombinant derived from an endemic Chinese PRV strain and a Bartha-like strain (Ye et al., 2016). Experimental studies have shown that Bartha-K61 can induce protection against the SC strain but not against the new variants (Luo et al., 2014). The SC strain has genomic regions similar to the Bartha strain, so it has been hypothesised that these similar regions may account for the capacity of the Bartha strain vaccine to protect against the SC strain, but not the new
variants, and that this difference in protective immunity may have permitted the new variants to
circulate in pig herds and cause disease (Ye et al., 2016)

3.3 Feline herpesvirus-1

Recombination between FeHV-1 isolates has been demonstrated in vitro (Fujita et al., 1998), but
only one study has investigated natural recombination in FeHV-1 using NGS (Vaz et al., 2016b).
Feline herpesvirus-1 is the aetiological agent of feline viral rhinotracheitis and also a common cause
of ocular lesions in cats (Maes, 2012). Inactivated and attenuated vaccines are used widely, but do
not prevent infection (Jas et al., 2009). Previous studies using techniques such as restriction
endonuclease digestion of the genome, and analysis of partial genomic sequences suggested low
levels of diversity among several isolates (Maeda et al., 1995). Comparison of the whole genomes
of 24 historical and contemporary FHV-1 clinical isolates and 2 US-origin commercial vaccine
viruses in use worldwide confirmed that FeHV-1 isolates are highly homogeneous and has revealed
no evidence of recombination (Vaz et al., 2016b). This is the first alphaherpesvirus in which
recombination has been shown to occur under experimental (in vitro) conditions, but not under
natural in vivo conditions. Low rates of FeHV-1 recombination in vivo have been hypothesised
previously, as FeHV-1 is more homogeneous than other alphaherpesviruses (Fujita et al., 1998), but
analyses of a larger number of FeHV-1 clinical isolates, from more diverse geographical regions are
required in order to fully assess recombination during natural FeHV-1 infection.

4. Natural recombination in avian alphaherpesviruses

Natural recombination has been described in infectious laryngotracheitis virus (ILTV) and Marek’s
disease virus type 1 (MDV-1, also called Gallid herpesvirus-2, GaHV-2) (Hughes and Rivailler,
2007; Lee et al., 2013; Lee et al., 2012) (Table 3). Marek’s disease virus induces T cell lymphomas
in susceptible birds (Morrow and Fehler, 2004), while ILTV causes upper respiratory tract disease
in chickens (Garcia et al., 2013). Both diseases are highly contagious and cause economic losses in poultry industries worldwide. Live attenuated vaccines are widely used to help control both these diseases.

4.1 Marek’s disease virus

Four complete GaHV-2 genome sequences (CVI988, GA, Md5 and Md11) were compared in 2007 and genes with unusually high degrees of synonymous divergence were identified, suggesting the past homologous recombination events (Hughes and Rivailler, 2007). This study identified three clusters of orthologous genes based on their patterns of synonymous substitutions in order to use them for further recombination analysis (Hughes and Rivailler, 2007). Eight loci within the four GaHV-2 genomic sequences were highly homogenous, suggesting homologous recombination between the vaccine strain CVI988, the highly virulent field strain (Md5) and the virulent Md11 strain. Additionally, phylogenetic analyses of the GA, Md5 and Md11 strains found that the virulent GA strain generally clustered separately from the highly virulent Md5 and Md11 strains, but further analyses of the UL 49.5 and RL ORF12 genes detected a high level of homogeneity between the GA, Md5 and Md11 strains, suggesting that recombination had resulted in the transfer of virulence factors between these strains. The approach used in this study to detect recombination differed from those used for other alphaherpesviruses, such as HSV-1, HSV2, VZV, EHV-1, EHV-4, FeHV-1 and ILTV (Bowden et al., 2004; Hughes and Rivailler, 2007; Kolb et al., 2013; Kolb et al., 2015; Lee et al., 2013; Lee et al., 2012; Norberg et al., 2015; Norberg et al., 2007; Norberg et al., 2006; Norberg et al., 2011; Peters et al., 2006; Szpara et al., 2014; Vaz et al., 2016a; Vaz et al., 2016b). Instead, the method used was extrapolated from those used to detect sites of homologous recombination within bacterial genomes. This approach examines the synonymous substitution distribution patterns among orthologous protein coding genes, with higher synonymous substitutions per site providing evidence of recombination (Hughes and Langley, 2007).
Analyses of GaHV-2 genome sequences for evidence of recombination, using methods similar to those used for other alphaherpesviruses, would help to further explore these findings. To this end, we examined the 15 GaHV-2 genome sequences publicly available at NCBI database (Table 4) for recombination using the SplitsTree 4 software and RDP4 software packages, as detailed previously (Vaz et al., 2016a; Vaz et al., 2016b). These analyses revealed evidence of recombination within the unique short region of the GaHV-2 genome using the SplitsTree 4 software (Figure 1C) (Huson and Bryant, 2006) and in all regions of the GaHV-2 genome using RDP4 software (Table 5). Together these results provide evidence of recombination in GaHV-2 but further study into the importance of recombination for GaHV-2 evolution and genome diversification is warranted, including examination of a larger number of GaHV-2 field isolates.

4.2 Infectious laryngotracheitis virus

Natural recombination in ILTV was first described by our laboratory in the context of attenuated vaccine use in Australia (Lee et al., 2012). This study provided clear evidence of natural recombination in ILTV, and also demonstrated safety concerns associated with the use of live attenuated alphaherpesvirus vaccines, a risk that had previously only been hypothesised. Prior to the detection of ILTV recombinants, two new genotypes of ILTV were shown to be dominant in Australia. These new genotypes, named as class 8 and 9 ILTV, had similar PCR-RFLP patterns, and clustered close to the Class 7 genotype (which includes the Serva vaccine strain) (Blacker et al., 2011). These studies, together with similar findings around the world led to the hypothesis that live attenuated ILTV vaccines could displace wild type strains and cause outbreaks of disease (Garcia and Riblet, 2001; Graham et al., 2000). However, the subsequent whole genome sequencing studies showed that natural recombination between the vaccine strains in use in Australia was responsible for the rise of the virulent Class 8 and 9 ILTV strains (Lee et al., 2012). These conclusions were supported by the use of the BootScan algorithm within the SimPlot program, which revealed the locations of breakpoints for intra-species recombination events involving the Serva and Australian...
origin SA2 and A20 vaccine strains (Lee et al., 2012). These findings echoed earlier studies that showed that recombination between two attenuated HSV-1 strains could generate more virulent strains in a mouse model of infection (Javier et al., 1986). In order to further investigate natural recombination in ILTV, full genome sequence data of other Australian ILTV isolates, along with full genome sequences of isolates from the US, were compared. The analyses revealed extensive recombination networks between ILTV isolates from both Australia and the US, and also uncovered new phylogenetic relationships between isolates (Lee et al., 2013). The importance of recombination in the biology and epidemiology of ILTV was then demonstrated further in 2016 in a study showing that a new virulent genotype of ILTV (Class 10 ILTV) had emerged in Australian poultry flocks as a result of recombination and had become dominant in some geographical areas (Agnew-Crumpton et al., 2016). These studies that have detected and characterised natural recombination in ILTV have provided new insights into the epidemiology of the disease caused by this virus and have explained the continuing occurrence of disease outbreaks associated with novel viral genotypes in Australia.

5 Conclusions

Alphaherpesviruses have been shown to display a high rate of recombination in vitro and in vivo under experimental conditions. However, under natural conditions, detection of recombination varies from limited or absent, in FeHV-1 and EHV-1 (Vaz et al., 2016a; Vaz et al., 2016b) to widespread, in HSV-1, EHV-4 and ILTV (Kolb et al., 2015; Lee et al., 2013; Lee et al., 2012; Szpara et al., 2014; Vaz et al., 2016a). These findings suggest that the contribution that recombination makes to genomic diversification and evolution in alphaherpesviruses varies across the different virus species. This highlights the importance of complementing studies that examine recombination in experimental settings with studies that look into recombination in field isolates from naturally infected hosts. The most comprehensive studies in this area have included analyses
of a large number of historical and contemporary whole genome sequences of field isolates from
diverse geographical regions.

Most studies of natural alphaherpesvirus recombination have focused on human alphaherpesviruses.
However, attenuated herpesvirus vaccines are used in both human medicine (against VZV) and
veterinary medicine. Their use is particularly widespread in livestock species, poultry and pets.
Recombination involving vaccine strains has been described in VZV, MDV (GaHV-2), ILTV
(GaHV-1) and PRV, but the recombination events in these virus species have differed in their
nature and consequences. In ILTV, two vaccine strains recombined to produce a virulent
recombinant that became a dominant field strain (Lee et al., 2012). In PRV, recombination occurred
between a vaccine strain and a field strain, potentially contributing to vaccine-induced selection and
protection of another, genetically less similar, field strain (Ye et al., 2016). These studies provide
examples of how vaccines and recombination can have an impact on viral evolution and alter the
selection pressure on a viral population to result in greater dissemination of more pathogenic
viruses. As live attenuated vaccines are favoured in veterinary medicine, their impact on viral
ecology and evolution should be evaluated and monitored at a population level. Targeted
monitoring of recombination after the introduction of new vaccine strains would be helpful for
detecting the rise of new, potentially more virulent strains. Consideration of recombination risks in
the assessment of vaccine safety during the process of registration could also be advantageous.
Currently, natural recombination has not been assessed in BoHV-1 or herpesvirus of turkeys (HVT)
but live vaccines using these viruses are in widespread use in the cattle and poultry industries,
respectively, with the latter being used as vaccine vector to express exogenous proteins from other
avian pathogens (Kapczynski et al., 2015; Li et al., 2011; Roh et al., 2016). Examining natural
recombination in these two viruses should be included in future research in order to determine
potential risks to animal health.

Over the last 10 years the development of new more sophisticated and accessible NGS techniques,
along with advances in computational and statistical analysis, has dramatically enhanced the study
of natural recombination in alphaherpesviruses. Detection of recombination using more than one approach is recommended and can now be achieved using a number of different bioinformatic analysis software. Commonly used programs include the Recombination Detection Program (RDP), SplitsTree software, the Genetic Algorithm Recombination Detection (GARD) program and SimPlot software. The latest version of RDP (RDP4) was made available in 2015 (Martin et al., 2015) and includes nine non-parametric recombination detection methods (Martin et al., 2010). SplitTree software uses phylogenetic and reticulate networks, in addition to the pairwise homology test (PHI test), to search for the presence of recombination in a given set of aligned sequences (Huson and Bryant, 2006). The GARD program searches for evidence of segment-specific phylogenies. GARD is a likelihood-based model selection procedure that searches multiple sequence alignments for evidence of recombination breakpoints and identifies putative recombinant sequences (Kosakovsky Pond et al., 2006). SimPlot allows the analysis of sequence alignments, and searches for breakpoint locations. This program ignores sites containing gaps, and produces a similarity plot identifying the sequence position and the similarity value at each point in the sequence (Lole et al., 1999). The application of more than one method is desirable to increase the robustness and consistency of the results, since a true recombination event should be independent of the method of analysis.

Sequence quality is also crucial to the accurate detection of recombination events in full genome sequences. Consideration of a measurement of quality such as Phred score and avoiding low sequence quality and parental sequence uncertainty is strongly recommended, since detection of breakpoint locations, and thus recombination, may be unreliable in low quality sequences (Posada, 2002; Posada and Crandall, 2001). Inclusion of detailed information describing the methods used in recombination analyses is also desirable, specifically in regard to the preparation of the sequences that are used for recombination analysis such as the trimming of short sequence repeats (SSR), tandem repeat regions (TRR), and terminal repeated regions (TR). It is recommended to remove these sequences from analyses as they can bias recombination analysis (Dutch et al., 1995; Lee et
Finally, studies focused on natural herpesvirus recombination should aim to include high quality information about the samples and virus isolates so that the results from recombination analyses can be considered in the context of relevant clinical and epidemiological information. Pairing recombination analyses with an understanding of the epidemiology and pathogenesis of the viruses offers the greatest potential to understand the importance of recombination and the role that it may be playing in virus evolution. Indeed, some of the most important findings relating to recombination and the involvement of attenuated vaccines have come from studies that have integrated recombination, epidemiological and viral pathogenesis. Maintaining such a focus in future studies will be important for achieving a comprehensive understanding of alphaherpesvirus recombination.

This review has highlighted the significance of natural recombination in many viruses of importance to human and veterinary medicine and has demonstrated the power of using full genome sequencing and sequence analysis to examine natural herpesvirus recombination. It is likely that continued advances in technology and methodologies, further reductions in the cost of NGS techniques and improved bioinformatics tools to assess recombination will enable further developments in the field of recombination, in particular by facilitating the affordable examination of increasing numbers of clinical isolates from diverse widespread geographical regions.

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Conflict of interest

The authors declare there is no conflict of interest in submission of this paper.

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**Figure captions:**

**Figure 1.** Recombination network trees generated using SplitsTree4 from alignment of 15 publically available GaHV-2 genome sequences on Genbank. **A)** Alignment of complete genomes excluding the terminal sequence repeats. **B)** Internal repeat region. **C)** Unique short region. The multiple reticulate networks indicate recombination events between the isolates. The bar indicates the rate of evolution in sequence substitution per site. *P* values for the PHI test for detecting recombination, as implemented in SplitsTree4, are shown and were highly significant for the complete genome and for the unique short region. **D)** Unique long region. Details about the nucleotide sequences used in these analyses are shown in Table 4.
<table>
<thead>
<tr>
<th>Year</th>
<th>Summary of study findings</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>2004</td>
<td>Evidence of recombination in partial genome sequences (4% of the genome) from 14 clinical samples from UK compared with samples from Seoul and South Korea.</td>
<td>Bowden et al., 2004</td>
</tr>
<tr>
<td>2004</td>
<td>Evidence of recombination in partial genome sequences (2.3% of the genome) in 28 clinical samples from Sweden.</td>
<td>Norberg et al., 2004</td>
</tr>
<tr>
<td>2011</td>
<td>Whole genome sequencing and analysis of seven clinical isolates shows one isolate had variable phylogenetic features, potentially due to recombination.</td>
<td>Kolb et al., 2011</td>
</tr>
<tr>
<td>2013</td>
<td>Widespread recombination was detected following whole genome sequencing of 31 clinical isolates from diverse geographical regions.</td>
<td>Kolb et al., 2013</td>
</tr>
<tr>
<td>2014</td>
<td>Widespread recombination was found following whole genome sequencing of 20 field strains obtained from China, Japan, Kenya and South Korea and comparison with those available from the US, Europe and Japan.</td>
<td>Szpara et al., 2014</td>
</tr>
<tr>
<td>2007</td>
<td>Analysis of partial genome sequences (3.5% of the genome) from 47 diverse clinical isolates shows HSV-2 has fewer genogroups than HSV-1.</td>
<td>Norberg et al., 2007</td>
</tr>
<tr>
<td>2015</td>
<td>Whole genome sequencing and analysis of six clinical isolates, along with analysis of two other available genome reveals evidence of recombination.</td>
<td>Kolb et al., 2015</td>
</tr>
<tr>
<td>2015</td>
<td>Analysis of 34 near complete genome sequences from clinical isolates from diverse geographical regions shows that recombination is present but is less frequent than in HSV-1.</td>
<td>Newman et al., 2015</td>
</tr>
<tr>
<td>2003</td>
<td>Analysis of SNP patterns from geographically diverse isolates classified VZV into four major groups and showed evidence of potential recombination.</td>
<td>Wagenaar et al., 2003</td>
</tr>
<tr>
<td>2004</td>
<td>Analysis of SNP patterns from geographically diverse isolates determined three major groups and showed evidence of potential recombination in one group/</td>
<td>Loparev et al., 2004</td>
</tr>
<tr>
<td>2006</td>
<td>Whole genome sequencing of 11 representative isolates from North America identified recombination potentially involving the VZV vaccine strain.</td>
<td>Peters et al., 2006</td>
</tr>
<tr>
<td>2011</td>
<td>Whole genome sequencing of a Russian clinical isolate showed significant intra-clade recombination events</td>
<td>Norberg et al., 2011</td>
</tr>
<tr>
<td>2015</td>
<td>Whole genome sequences of 37 isolates from diverse geographical regions confirmed that viruses in the Japanese vaccine-like group consistently display higher levels of recombination.</td>
<td>Norberg et al., 2015</td>
</tr>
</tbody>
</table>

* Live attenuated vaccine is in use
Table 2. Timeline and summary of key studies examining natural recombination in other (non-human) mammalian alphaherpesviruses.

<table>
<thead>
<tr>
<th>Year</th>
<th>Summary of study findings</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>2005</td>
<td>Analysis of partial genome sequence detected the first natural inter-species recombination reported between EHV-1 and EHV-4.</td>
<td>Pagamjav et al., 2005</td>
</tr>
<tr>
<td>2012</td>
<td>Analyses of partial genome sequences of an isolate from a polar bear in a zoo revealed inter-species recombination between EHV-1 and EHV-9</td>
<td>Greenwood et al., 2012</td>
</tr>
<tr>
<td>2016</td>
<td>Whole genome sequencing of 11 EHV-1 and 14 EHV-4 isolates from Australia and New Zealand, and comparison with other available genomes, showed widespread recombination in EHV-4 but not in EHV-1.</td>
<td>Vaz et al., 2016a</td>
</tr>
<tr>
<td>2016</td>
<td>Whole genome sequencing of isolates from China demonstrated recombination involving a vaccine-like strain, potentially altering selection pressures in vaccinated pig populations</td>
<td>Ye et al., 2016</td>
</tr>
<tr>
<td>2016</td>
<td>Analyses of 24 whole genome sequences from clinical samples and 2 genome sequences of US origin vaccines found no evidence of recombination.</td>
<td>Vaz et al., 2016b</td>
</tr>
</tbody>
</table>

* Live attenuated vaccine is in use
Table 3. Timeline and summary of key studies examining natural recombination in avian alphaherpesviruses.

<table>
<thead>
<tr>
<th>Year</th>
<th>Summary of study findings</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>2007</td>
<td>Four whole genome sequences were analyzed and recombination was identified between a vaccine strain and a highly virulent field strain</td>
<td>Hughes and Rivallier et al., 2007</td>
</tr>
<tr>
<td>2012</td>
<td>Whole genome sequence analyses of newly emerged field isolates found that two attenuated vaccine strains had recombined to generate virulent viruses.</td>
<td>Lee et al., 2012</td>
</tr>
<tr>
<td>2013</td>
<td>Whole genome sequence analyses of current and historical isolates in Australia and the US revealed extensive recombination networks</td>
<td>Lee et al., 2013</td>
</tr>
<tr>
<td>2016</td>
<td>A newly emerged virulent field strain in Australia was sequenced and shown to be a recombinant virus.</td>
<td>Agnew-Crumpton et al., 2016</td>
</tr>
</tbody>
</table>

* Live attenuated vaccine is in use
Table 4. Publically available full genome sequences of GaHV-2 used in recombination analyses.

<table>
<thead>
<tr>
<th>Isolate (GenBank accession number)</th>
<th>Year of isolation (Reference)</th>
<th>Country</th>
<th>Comments</th>
<th>Genome sequence reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>GA (AF147806)</td>
<td>1964 (Eidson and Schmittle, 1968)</td>
<td>USA</td>
<td>Isolated from ovarian tumour</td>
<td>(Lee et al., 2000)</td>
</tr>
<tr>
<td>Md5 (AF243438)</td>
<td>1980 (Witter et al., 1980)</td>
<td>USA</td>
<td>Very virulent isolate from spleen of commercial broilers</td>
<td>(Tulman et al., 2000)</td>
</tr>
<tr>
<td>Md11 (AY510475)</td>
<td>1980 (Witter et al., 1980)</td>
<td>USA</td>
<td>Isolated and maintained in duck embryo fibroblast</td>
<td>(Niikura et al., 2006)</td>
</tr>
<tr>
<td>CVI988 (DQ530348)</td>
<td>1972 (Rispens et al., 1972)</td>
<td>Worldwide use</td>
<td>Vaccine used since 1990</td>
<td>(Spatz et al., 2007a)</td>
</tr>
<tr>
<td>RB-1B (EF523390)</td>
<td>1982 (Schat et al., 1982)</td>
<td>USA</td>
<td>Highly oncogenic</td>
<td>(Spatz et al., 2007b)</td>
</tr>
<tr>
<td>CU-2 (EU499381)</td>
<td>1973 (Smith and Calnek, 1973)</td>
<td>USA</td>
<td>Mildly virulent</td>
<td>(Spatz and Rue, 2008)</td>
</tr>
<tr>
<td>814 (JF742597)</td>
<td>1980</td>
<td>China</td>
<td>Isolated from healthy chickens</td>
<td>(Zhang et al., 2012)</td>
</tr>
<tr>
<td>LMS (JQ314003)</td>
<td>2007</td>
<td>China</td>
<td>Isolated from broilers with severe disease</td>
<td>(Cheng et al., 2012)</td>
</tr>
<tr>
<td>GX0101 (JX844666)</td>
<td>2001 (Zhang et al., 2012)</td>
<td>China</td>
<td>Isolated from layers with severe tumours</td>
<td>(Su et al., 2012)</td>
</tr>
</tbody>
</table>
Table 5. Recombination breakpoint analysis of GaHV-2 genome sequences using RDP4

<table>
<thead>
<tr>
<th>Genome region</th>
<th>Breakpoint (in alignment)</th>
<th>Possible viruses involved in recombination event</th>
<th>Method of breakpoint detection in RDP4 software</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Breakpoint beginning 99% CI*</td>
<td>Breakpoint ending 99% CI*</td>
<td></td>
</tr>
<tr>
<td>Internal/terminal</td>
<td>18291 – 19335</td>
<td>19926 - 21087</td>
<td>R: GA, M: unknown, m: GX0101</td>
</tr>
<tr>
<td>repeat</td>
<td></td>
<td></td>
<td>GENECONV, MaxChi, 3Seq.</td>
</tr>
<tr>
<td></td>
<td>51568 – 73297</td>
<td>108359 – 3016</td>
<td>GENECONV, Bootscan, MaxChi, Chimaera, 3Seq.</td>
</tr>
<tr>
<td></td>
<td>91057 – 58003</td>
<td>91057 - 58003</td>
<td>R: GX0101, M: 814, m: unknown</td>
</tr>
<tr>
<td></td>
<td>82371 – 93738</td>
<td>93740 – 101624</td>
<td>GENECONV, MaxChi, SiScan, 3Seq.</td>
</tr>
<tr>
<td>Unique short</td>
<td>2289 – 3999</td>
<td>5959 – 7480</td>
<td>R: CVI988, M: LMS, m:GX0101</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>GENECONV, MaxChi, Chimaera, SiScan, 3Seq.</td>
</tr>
</tbody>
</table>

*CI = confidence interval
A) Complete genome ($p = 9.751\times10^{-9}$)

B) Internal repeat ($p = 0.05766$)

C) Unique short ($p = 0.002421$)

D) Unique long ($p = 0.2554$)
Author/s: Loncoman, CA; Vaz, PK; Coppo, MJC; Hartley, CA; Morera, FJ; Browning, GF; Devlin, JM

Title: Natural recombination in alphaherpesviruses: Insights into viral evolution through full genome sequencing and sequence analysis

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