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Research paper

Highlighting priority areas for bovine viral diarrhea control in Italy: A phylogeographic approach

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ABSTRACT

The prevalence and genetic diversity of bovine viral diarrhea virus (BVDV) in a geographic area are largely influenced by live animal trade and management practices. Despite control and eradication programs currently underway in several European countries, the risk of BVDV spread within and among countries is still present. BVDV-1 is the predominant type circulating in European cattle population. In this study, a phylogeographic analysis was applied to the BVDV-1 highest prevalent subtypes in Italy to reconstruct the origin and spatial-temporal distribution and to trace main viral flows between different locations to highlight priority areas for BVDV control. A comprehensive dataset of BVDV-1b (n = 173) and 1e (n = 172) 5′ UTR sequences was analysed, including both novel and published sequences from Italy and from European countries bordering and/or with commercial cattle flows with Italy. A common phylogeographic pattern was observed for BVDV-1b and 1e subtypes: interspersion from multiple Italian areas and European countries was widespread until the end of the last century, whereas significant local clusters were observed starting from 2000. These findings support a continuous viral flow among different areas over long time scales with no evidence of significant geographical structure, while local transmission networks are limited to more recent years. Northern Italy has been confirmed as the area of origin of the main clades of both BVDV subtypes at national level, acting both as a crucial area for introduction and a maintenance source for other areas. Piedmont, Central and Southern Italian regions contributed to limited geographical distribution and local BVDV-1b and 1e persistence. On the whole, priority control measures for BVDV-1b and 1e in Italy should be focused on: i) implementation of BVDV systematic control in all Northern Italian regions to break the viral flow from larger to smaller animal populations; and ii) breaking the dynamics of infections in regions with self-maintenance of BVDV by voluntary control programs.

1. Introduction

Bovine viral diarrhea virus (BVDV) is a widespread pathogen of cattle. Occurrence of disease, economic impact, its wide distribution and inclusion of BVDV in the OIE list increased the awareness towards this disease (Gunn et al., 2005). Control and eradication programs are underway in several European countries and Scandinavian countries are currently either free, or almost free from BVDV (Ståhl and Alenius, 2012).

Nowadays, most of the countries from the Mediterranean basin show an endemic diffusion of BVDV infection (Hurtado et al., 2003; Billinis et al., 2005; Luzzago et al., 2014; Aslan et al., 2015) and are not applying any systematic control at national level. In Italy, regional mandatory eradication programs are restricted to Trentino Alto-Adige, bordering Austria, whereas voluntary control programs are applied in few other northern regions (Piedmont, Veneto, Friuli-Venezia Giulia). Italy showed a high BVDV genetic diversity compared to other countries and the most likely explanation is livestock trade from European countries and among Italian regions (Luzzago et al., 2012; Giammarioli et al., 2015).

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BVDV belongs to the *Pestivirus* genus of the *Flaviviridae* family. Genetic typing of BVDV isolates distinguishes two recognized species (BVDV-1 and BVDV-2) and a putative third bovine species, referred to as HoBi-like pestivirus or BVDV-3 (Liu et al., 2009). To date, 17 BVDV-1 subtypes have been identified and four additional novel subtypes have been proposed and revised (BVDV-1a to BVDV-1u). Such diversity within this species raised concern related to the emergence and spread of new BVDV variants, with possible implications for animal health and disease control (Giammarioli et al., 2015; Yeşilbağ et al., 2017).

The BVDV type and subtype prevalence in a given geographic area has been largely influenced by live animal trade and management practices. Long distance transmission between regions and countries is mainly related to livestock trade of BVDV persistently infected (PI) animals or dams carrying a PI foetus (Lindberg and Alenius, 1999). The most recent evidences of BVDV spread was documented in England and Wales, which occurred due to restocking with cattle from continental Europe (Strong et al., 2013), and in Germany and Netherlands, following animal trade during the BVDV-2 outbreaks (Gethmann et al., 2015). Therefore, the risk of BVDV spread within and among countries is still high and present.

Genetic characterization of isolates and phylogeographical analysis gives a unique opportunity to trace routes of infection. In fact, phylogeography combines spatial and temporal analyses of isolates sampled at known times in different areas to reveal location and time of origin of infections and flow of geographic spread (Pybus and Rambaut, 2009; Zehender et al., 2013). The methods currently used of phylogeographical reconstruction are inherently limited by the availability of sequences that respect the minimum criteria of inclusion, namely time of collection and location of origin of the strains.

The BVDV 5′ UTR region is a non-coding region of the genome, which has been used extensively for diagnostic purpose and virus typing (Vilcek et al., 2004). Therefore, a large number of 5′ UTR sequences are available and publicly accessible from databases, with additional information on the collection date and geographical origin of the isolates necessary for advanced phylogenies. Phylogeographic analysis built on the 5′ UTR sequence are informative (Luzzago et al., 2012) especially over long time scales, such as decades, while regions with higher variability (e.g., Npro and E2 genes) allow resolution of phylogeny over shorter time scales (Chernick et al., 2014).

The aim of this study was to reconstruct the origin and spatio-temporal distribution of the highest prevalent subtypes in Italy (Luzzago et al., 2014) and in several European countries (Tajima et al., 2001; Jackova et al., 2008; Hornberg et al., 2009; Kuta et al., 2013) to highlight priority actions to be introduced to control BVDV. A phylogeographic analysis of a comprehensive dataset of BVDV-1b and 1e 5′ UTR sequences was performed, including both novel and published sequences from Italy and other European countries bordering and/or with commercial cattle flows with our country.

2. Materials and methods

2.1. Samples and subtype assignment

Samples sent to laboratories for routine testing because of suspected BVDV infection were used, therefore no animals were specifically sampled to perform this study. Twenty-six positive samples were obtained from 26 Italian cattle herds and were characterized by RT-PCR and sequencing as previously described (Luzzago et al., 2014). The sequences were classified by alignment with BVDV-1 reference strains retrieved from GenBank by using Clustal X with BioEdit software version 7.0 (freely available at http://www.mbio.ncsu.edu/bioedit/bioedit.html). Phylogeny was estimated by the neighbor-joining algorithm (NJ) and the maximum likelihood (ML) method with 1000 bootstrap replicates. The sequences of subtype -1b and 1e were selected and included in the following comprehensive datasets.

2.2. BVDV-1b and 1e datasets

Two datasets of sequences for each BVDV subtype were generated.

The first included Italian sequences only and was obtained aligning the novel sequences from this work with other Italian sequences retrieved from published peer-reviewed journals and characterized by locality and year of sampling collection. Inclusion criteria allowed to retrieve BVDV-1b and BVDV-1e sequences from Northern Italian areas: Piedmont (n = 102), Lombardy (n = 80), Emilia-Romagna (n = 26), Veneto (n = 13) (Falcone et al., 2003; Luzzago et al., 2001, 2012, 2014); from Central areas (n = 12) (Giammarioli et al., 2008; Luzzago et al., 2014) and Southern Italy (n = 19) (Cannella et al., 2012; Luzzago et al., 2012, 2014). The sampling dates ranged from 1995 to 2013 for BVDV-1b and from 1996 to 2013 for BVDV-1e (Table S1).

A second dataset (Italian plus European) was generated for each subtype by implementing Italian sequence' datasets with BVDV-1b and 1e sequences from European cattle, retrieved from public database, restricting the geographic localization to countries bordering Italy and/or linked by commercial flows with Italy (https://www.vetinfo.sanita.it). Inclusion criteria for European sequences was availability of sample collection year. Moreover, in a group of sequences from the same Polish herd a single sequence was selected in case of 100% genetic identity. A total of 34 BVDV-1b and 38 BVDV-1e sequences were obtained from the following countries: Austria (n = 8) (Hornberg et al., 2009; La Rocca and Sandvik, 2009), France (n = 43) (Jackova et al., 2008; La Rocca and Sandvik, 2009), Germany (n = 4) (Tajima et al., 2001), Poland (n = 13) (Kuta et al., 2013), Slovenia (n = 2) (Toplak et al., 2004), Switzerland (n = 2) (Bachofen et al., 2008). The sampling dates of European sequences ranged from 1960 to 2011 for BVDV-1b and from 1994 to 2006 for BVDV-1e (Table S1).

2.3. Phylogeographic and phylodynamic analysis

2.3.1. Likelihood mapping analysis

The phylogenetic signal of each dataset was investigated by means of the likelihood-mapping analysis of 10,000 random quartets generated using TreePuzzle (Schmidt et al., 2002). All of the three possible unrooted trees for a set of four sequences (quartets) randomly selected from the dataset were reconstructed using the maximum likelihood (ML) approach and the selected substitution model. The posterior probabilities of each tree were then plotted on a triangular surface so that the dots representing the fully resolved trees fell at the corners and those representing the unresolved quartets in the centre of the triangle (star-tree) (Schmidt et al., 2002). Using this strategy, described in detail elsewhere (Strimmer and von Haeseler, 1997), the data were considered unreliable for phylogenetic inference when > 30% of the dots were in the centre of the triangle.

2.3.2. Phylogenetic reconstruction

The analysis was performed for both BVDV-1 subtypes. The best-fitting nucleotide substitution model was estimated by means of JModelTest (Posada, 2008), and selected a HKY model (Hasegawa et al., 1985) with gamma-distributed rates among sites. The phylogenetic tree, model parameters, evolutionary rates and population growth were co-estimated using a Bayesian Markov chain Monte Carlo (MCMC) method implemented in the BEAST v.1.8.0 package (Drummond et al., 2012).

Statistical support for specific clades was obtained by calculating the posterior probability of each monophyletic clade. As coalescent priors, four simple parametric demographic models (constant population size, and exponential, expansion and logistic population growth) and a piecewise-constant model, the Bayesian skyline plot (BSP) under both a strict and a relaxed (uncorrelated log-normal) clock were compared (Drummond et al., 2012).

Two independent MCMC chains were run for 150 million generations with sampling every 15,000th generation for BVDV-1b dataset