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Phylodynamic and phylogeographic reconstruction of porcine reproductive and respiratory syndrome virus (PRRSV) in Europe: Patterns and determinants

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Abstract

Porcine reproductive and respiratory syndrome (PRRS) is among the most devastating diseases affecting the pig industry. Despite vaccines having been available for decades, the remarkable genetic variability of this virus, leading to poor cross-protection, has limited their efficacy, and other measures must be adopted to effectively control the viral circulation. Some recent studies have investigated the factors involved in viral spreading and persistence, at least at the local level. However, despite the topic's relevance, no statistically grounded evidence is currently available evaluating the variables more involved in porcine reproductive and respiratory syndrome virus (PRRSV) epidemiological success at a broader scale, such as the European scale. In the present study, an extensive phylodynamic and phylogeographic analysis was performed on more than 1000 ORF5 sequences to investigate the history, dynamics and spreading patterns of PRRSV within European borders. Moreover, several potential predictors, representative of swine population features and trade, human population, economy and geographic characteristics, were evaluated through a specifically designed generalized linear model (GLM) to assess their weight on viral migration rate between countries over time. Although pig stock density, mean PRRSV strain genetic diversity, investments in agriculture (including a likely role of vaccination) and farmer education were involved to a certain extent, the major determinant was proven to be by far the live pig trade. Providing a robust depiction of PRRSV European molecular epidemiology patterns and determinants, the present study could contribute to a more rational allocation of limited resources based on an effective prioritization of control measures.

KEYWORDS
control, Europe, GLM, ORF5, phylodynamics, phylogeography, PRRSV

1 | INTRODUCTION

Porcine reproductive and respiratory syndrome (PRRS) is among the most challenging and economically damaging diseases affecting the pig industry (Nieuwenhuis et al., 2012; Segalés & Mateu, 2012). It is caused by two viral species, *Betaarterivirus suid* 1 and *Betaarterivirus suid* 2, belonging to the genus *Betaarterivirus*, family *Arteriviridae* (https://talk.ictvonline.org/taxonomy, accessed on 10 November 2021). These viruses are historically and commonly known as porcine reproductive and respiratory syndrome virus 1 (PRRSV-1) and porcine reproductive and respiratory syndrome virus 2 (PRRSV-2). They are characterized by a single-stranded, positive-sense RNA (ssRNA(+)) genome of approximately 15 kb, encoding several proteins. Approximately three-quarters of the genome is occupied by ORF1a and ORF1ab, encoding 14 nonstructural proteins, while the terminal part consists of eight partially overlapping ORFs (ORF2a, ORF2b, ORF3, ORF4, ORF5, ORF5a, ORF6 and ORF7) (Dokland, 2010). Similar to other RNA viruses, PRRSV shows a high evolutionary rate, generating a plethora of variants on which genetic drift and selective pressures, particularly those induced by host immunity, can act, leading to the observed genetic, phenotypic and biological heterogeneity of circulating strains (Duffy, 2018; Murtaugh et al., 2010; Shi et al., 2010; Stadejek et al., 2013).

Variability in immunological features and cross-protection is probably the most investigated topic because of their practical implications (Mateu & Diaz, 2008). Several viral proteins have been demonstrated to be involved in the PRRSV immune response. However, the nucleocapsid (N) and especially glycoprotein 5 (GP5), encoded by ORF7 and ORF5, respectively, are by far the most studied. Because of the high genetic variability of ORF5, this gene is the most frequently sequenced and used for molecular epidemiology studies.

Since its first description in 1987, PRRS has been the target of intense research activity to understand its pathogenesis, epidemiology and potential control approach (Drew, 2011). Moreover, farmers and agricultural and pharmaceutical companies have made major efforts towards its control, developing and applying different direct and indirect control measures.

Nevertheless, such efforts are often frustrated, and adequate control of infection and disease is far from being achieved (Corzo et al., 2010; Fahrion et al., 2014). Vaccination is considered a cornerstone of the control, and different attempts have been made in terms of vaccine choice and administration strategies. Although variable success has been experimentally proven in several circumstances, proper and reliable protection can rarely be achieved in field conditions, largely because of the reduced cross-protection among highly heterogeneous strains, including vaccine strains (Kimman et al., 2009; Renukaradhya et al., 2015; Zhou et al., 2021).

Biosecurity is thus a fundamental complement to any vaccination plan, reducing the risk of new strain introduction and associated outbreaks. Moreover, a reduction in viral spreading and circulation should effectively contain the viral population size and thus its evolutive potential (Drew, 2011). In a recent study performed in Italy, the effect of both environmental and managerial variables, especially those related to pig flux and transportation, was demonstrated in PRRSV migration within and between pig companies (Franzo et al., 2021). Comparable results have previously been obtained in US studies, highlighting a common trend likely dictated by the similarities in the swine industry in developed countries (Arruda et al., 2017; Jara et al., 2021; Makau et al., 2021). Despite their practical interest, these results should be considered just one part of the puzzle, being based on a limited geographic area. The increasing economic and commercial connection among countries has largely shaped the epidemiology of several pathogens of both veterinary and human interest at a broader scale. Different studies have investigated the effect of various factors enhancing or hindering the spreading of such agents among locations. While human movement and animal trades have consistently been associated with the spread of several pathogens in different regions of the world, more conflicting results were obtained for other variables (He et al., 2022; Lemey et al., 2014; Magee et al., 2015; Nelson et al., 2015).

The spreading of PRRSV among countries has been largely demonstrated and contributes to increasing the within-country genetic variability and the consequent risk of low cross-protection and clinical outbreaks. However, the determinants of PRRSV circulation among European countries have never been formally investigated, even though a proper understanding of such a phenomenon could help infection control and favour better resource allocation. In the present study, an extensive phylodynamic study was performed based on a broad collection of ORF5 sequences obtained from PRRSV strains circulating in Europe to investigate their evolution, population dynamics and spreading patterns. Variables representative of farming, demographic, economic, social and population education features were also considered to investigate their relevance and contribution to such migration patterns.

2 | MATERIALS AND METHODS

2.1 | Data set

ORF5 sequences were downloaded from GenBank if 1) the sampling country and date were available, 2) the strains were collected in Europe and 3) the collection date was prior to 2019. The latter condition was necessary to reduce the bias and incorrect estimation of viral population size in recent years due to the limited sequence availability and restricted geographic origin, which would lead to an artificial reduction of estimated viral population size in the last part of the considered period.

All sequences were aligned using MAFFT (Standley, 2013), and their quality was evaluated. Poorly aligned sequences, those displaying unknown bases, premature stop codons or frameshift mutations and those closely related to vaccine strains were removed from the alignment. Recombination analysis was performed as described by Franzo et al. (2021) and recombinant strains were also excluded from further analysis. The presence of adequate phylogenetic and temporal signals was tested using the likelihood mapping approach implemented in

IQ-Tree (Nguyen et al., 2015) and the TempEst (Rambaut et al., 2016) program, respectively.

To exploit the impact of convenience-based sampling (causing a different sequence availability for each country) on population parameter estimates, the refined data set was reduced by randomly sampling without replacement up to 10 sequences for each country-year pair. Five data sets were generated, independently analysed and compared to assess the reliability and repeatability of the obtained results.

2.2 | Viral population dynamics and phylogeography

European PRRSV population parameters, including time to the most recent common ancestor (tMRCA), evolutionary rate and population dynamics variation over time, were jointly estimated on the five independent data sets using the Bayesian serial coalescent approach implemented in BEAST 1.10.4 (Suchard et al., 2018). The best nucleotide substitution model was selected based on the Bayesian Information Criterion (BIC) calculated using JModelTest2 (Darriba et al., 2012), while the molecular clock model was chosen based on Bayesian factor (BF) calculation obtained estimating the marginal likelihood of the evaluated models using the path sampling (PS) and stepping stones (SS) methods (Baele et al., 2012). The nonparametric Skygrid (Hill & Baele, 2019) model was selected to depict the variation in the relative genetic diversity (i.e., Effective population size × generation time; Ne × t) over time.

Strain migration among European countries was reconstructed using the discrete state phylogeographic approach described by Lemey et al. (2009) considering each collection country as a strain trait (Lemey et al., 2009). This model allows character mapping on a natural time scale under a molecular clock assumption and accounts for population size changes while integrating them over phylogenetic uncertainty. The Bayesian stochastic search variable selection (BSSVS) was also selected, which allowed the construction of a BF test that identifies the most parsimonious description of the phylogeographic diffusion process.

All parameters were jointly estimated in a Bayesian fashion using a 200 million generation Markov Chain Monte Carlo (MCMC) chain and sampling the population parameters and trees every 20,000 generations. Run performances were preliminarily summarized and evaluated using Tracer 1.7 after removing the first 20% of the data as burn-in. Run results were accepted only if the estimated sample size (ESS) was higher than 200 and the mixing and convergence, evaluated by visual inspection of the run's trace, were adequate. A maximum clade credibility (MCC) tree was obtained summarizing the tree posterior distribution using the Treeannotator suite of the BEAST package.

The viral spreading within Europe over time was analysed and displayed using SPREAD3 (Bielejec et al., 2016). Migration rates between country pairs were considered nonzero (i.e., statistically supported) when the respective BF, calculated with the same software, was greater than 10 in at least three out of five randomly generated data sets. The expected number of transitions, known as Markov jumps, between each pair of countries along the phylogenetic branches of the trees was also estimated (Minin & Suchard, 2008).

2.3 | Predictors of PRRSV strain exchange among countries

According to the implemented discrete state phylogeographic approach, viral migration between the K countries was modelled as a K•K rate matrix (Λ), with Λ_{ij} being the instantaneous relative transition rate from location i to j. Such rates can be parameterized as a log-linear function of various potential predictors (p) using a generalized linear model (GLM) (Lemey et al., 2014). Each potential predictor in the GLM is associated with a β_p coefficient, quantifying its effect size on Λ, and a binary indicator δ_p that determines its inclusion or exclusion as predictor in the model:

$$\log \Lambda_{ij} = \log(p_1) \times \beta_1 \times \delta_1 + \log(p_2) \times \beta_2 \times \delta_2 + \cdots + \log(p_n) \times \beta_n \times \delta_n$$

A BSSVS was used to explore the space of all potential predictor combinations and obtain the posterior probability of the indicator variable δ_p. Predictor inclusion support can thus be assessed through BF, estimated as the ratio of the posterior odds over the prior odds for predictor inclusion. In the present study, a Bernoulli prior probability distribution was set on these indicators, assigning a 50% prior probability on no predictors being included in the model. A predictor was considered statistically supported if BF>10.

Several potential predictors, representative of swine population features (i.e., number of animals, density), trade, human population (i.e., number of people, percentage of employment in agriculture, education, etc.), economy (per capita income, investments in agriculture) and geography (country size, minimum distance between country borders) were evaluated. Data were obtained from FAOSTAT and EUROSTAT archives (last accessed on 20/12/2021) or from country-specific databases when information was missing. Summary statistics representative of viral genetic variability were also considered. For country-specific measures, the same predictor was included for both origin and destination sites. Moreover, the sequence sample size for each country was included in the model to evaluate the effect of sampling intensity bias. The effect of sequencing availability was further tested by performing the analysis on the independent, randomly generated data sets. Additionally, sensitivity analysis was performed by randomly including a subset of the considered variables in the model. Finally, since a major increase in swine trade was observed in Europe after ~2000, independent models were fitted based on the predictor values featuring the two epochs defined by this cut-off.

3 | RESULTS

3.1 | European PRRSV population parameters

A total of 1055 sequences were maintained for the analysis, collected in the period 1991–2019 and originating from 23 countries (Supporting Information).
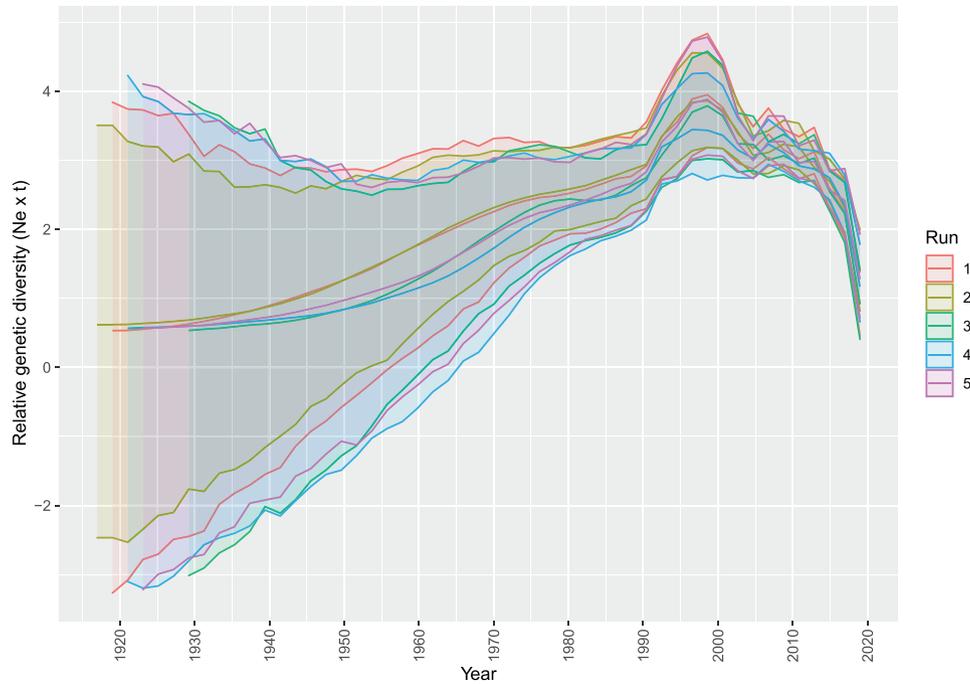
FIGURE 1 Plot reporting the mean relative genetic diversity (effective population size × generation time; Ne × t) of the European porcine reproductive and respiratory syndrome virus (PRRSV) population over time. This measure can be considered a proxy for the variation in PRRSV population size over time. The results of the five independent runs (obtained by randomly subsampling the whole sequence datasets) have been color-coded. 95HPD has been reported as a shaded area

The estimated evolutionary rate was consistent among all randomly generated and subsampled sequence data sets (mean: $3.01 \cdot 10^{-3}$; 95HPD: $2.24 \cdot 10^{-3} - 3.94 \cdot 10^{-3}$) (Figure S1). Similarly, the tMRCA was estimated approximatively in the first half of the 20th century data sets (mean: 1943.26; 95HPD: 1916.13−1964.88) (Figure S1).

When variation in population size over time was evaluated, a progressive increase in relative genetic diversity was observed from the estimated tMRCA to the 1990s, when a sharper peak became apparent, culminating approximatively in 2000 and then progressively decreasing until 2010 when a stabilization occurred. A final, marked, decrease was estimated in recent years (i.e., 2017–2019) (Figure 1).
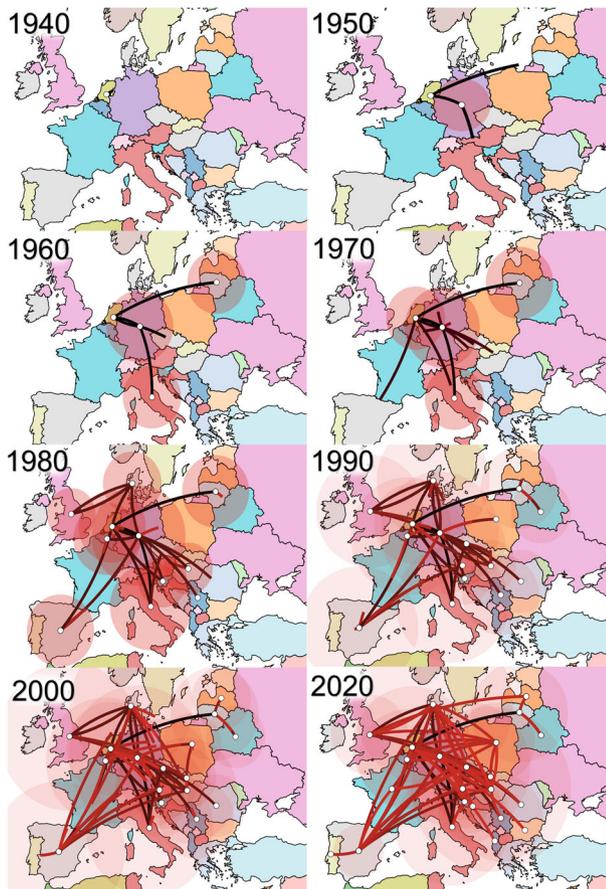
3.2 | Phylogeographic analysis

The reconstruction of PRRSV migration across Europe showed a complex and intricate pattern, especially after 2000, when most of the countries were affected and contributed to disease spreading to other countries (Figure 2). Nevertheless, despite these intricacies and some differences, the overall pattern was confirmed by all analysed sequence data sets and the consistency of adequately supported among countries' migration rates, regardless of the considered data set (Figure S2 and Figure 3). Overall, after the likely MRCA localized in Denmark, the virus spread to Eastern (Lithuania), Central (Germany) and Southern (Italy) Europe. Countries involved in this initial Northern-Southern axis took part in most of the further westward viral spreading. Thereafter, Western countries such as Spain, France and England participated in an inverse eastward viral flux. On the contrary, the virus

remained mostly confined to Eastern countries that, after viral introduction, made a limited contribution to its dispersal. Poland was the most relevant exception, particularly since the late 2000s. A certain role of Balkans countries in viral dispersal was also detected, especially from Serbia (late 1990s) and thereafter Croatia, which nevertheless involved mostly countries of the same area (i.e., formed-Yugoslavia). Accordingly, the reconstruction of state change count along the phylogenetic trees confirmed such a scenario, with a high density (Figure 4) of migrations (i.e., state changes) occurring among countries of Central Europe and between those and the rest of the continent. A much lower number of transitions originating from Eastern countries, regardless of the destination, was observed. An intermediate contribution was instead revealed for Western countries, which contributed to eastward spreading to Central and Eastern Europe, although their role as a viral source was lower than that of Central European countries. The only exception was represented by state changes within Western Europe, which had an intensity comparable to the importation from Central European countries (Figure 4).

The analysis of lineage through time confirmed the above-mentioned scenario and highlighted that after PRRSV introduction within a country, the virus persisted through the considered study period, although with certain fluctuations over time (Figure 5).

3.3 | Factors associated with PRRSV dispersal

The GLM-based analysis evaluating the relevance of different factors in affecting between-countries viral migration consistently detected live

4 | DISCUSSION

In 2004, health experts from around the world formulated the so-called Manhattan principles, a list of 12 recommendations for establishing a more holistic approach to prevent epidemic/epizootic diseases. Among the founding criteria, they emphasized that "It is clear that no one discipline or sector of society has enough knowledge and resources to prevent the emergence or resurgence of diseases in today's globalized world".

The present study attempts to follow these principles, integrating microbiology, genetics, demography, informatics and statistical data and methods in a common framework, aiming to unravel the dynamics and spreading determinants of PRRSV in Europe. Phylogeographic inference is a widely applied approach that enables the reconstruction of the geographic dispersion of a virus over time based upon the analysis of the genome sequence of strains whose sampling date and location are known (Lemey et al., 2009, 2010). Different extensions have also been developed to assess the impact of potential predictors on the geographic spread of viruses. There is little doubt that PRRS represents the single most important infectious disease for the pig industry (Drew, 2011), and there is a general notion that the intensification and globalization of the industry have contributed to the emergence and spread of PRRSV, as well as other swine pathogens (Segalés et al., 2013; VanderWaal & Deen, 2018). Therefore, recognizing the underlying drivers and patterns of such a process can allow their categorization and prioritization for public health actions (Semenza et al., 2016).

Not surprisingly, the present study demonstrated the high evolutionary rate of PRRSV strains in Europe, which perfectly match with previous estimates and previously known for RNA viruses (Duffy et al., 2008; Franzo et al., 2015; Franzo, Tucciarone et al., 2020). The estimated tMRCA largely anticipates the first reports of clinical outbreaks both in America and Europe (Semenza et al., 2016), demonstrating a long-lasting, subclinical circulation of this virus. Such a scenario is a common feature of several livestock infections, highlighting the pivotal relevance of farming conditions and productive system features in driving the emergence of overt clinical syndromes. The analysis of viral population dynamics supported this association. The slow but progressive increase in viral population size from tMRCA to the 1990s mirrors the progressive intensification of pig farming in Europe. Although the overall pig population trend featured a constant or even declining number, a parallel increase in farm size and productivity occurred (Marquer, 2010). Intensive high-density breeding systems can facilitate viral transmission within and among farms, in addition to enhancing animal susceptibility due to stressing conditions, genetic line selection, coinfections etc. An even sharper rise was observed since the first half of the 1990s, lasting approximately 10 years. Of note, a progressive increase in the live pig trade was observed in the same years in Europe, in concurrence with the foundation of the European single market. Accordingly, the phylogeographic analysis revealed that viral dispersal within Europe, although always present, became especially intense and complex since that period and persisted until today. Sampling and sequencing bias could have affected the migration pattern reconstruction and concealed a certain number of transmission events. However,

FIGURE 2 Phylogeographic reconstruction of porcine reproductive and respiratory syndrome virus (PRRSV) migration among European countries over time. Each picture represents a different decade. The edges connecting the countries have been color-coded from black to red based on the estimated age

pig trade as the only significantly associated factor (BF>100) regardless of the randomly generated data set (Figure S3). Accordingly, the conditional effect size (EF) deviated significantly from 0 for this variable only (i.e., EF was between 2.497 and 3.061, depending on the particular data set. A partial exception was represented by data set 5, where in addition to animal trade, a low education level of people involved in agriculture and pig stock density in the country of origin received adequate statistical support (BF = 31.15 and 12.8, respectively). A low education level had a negative contribution to strain migration (EF = −0.44), while a positive contribution (EF = 0.28) was estimated for pig density.
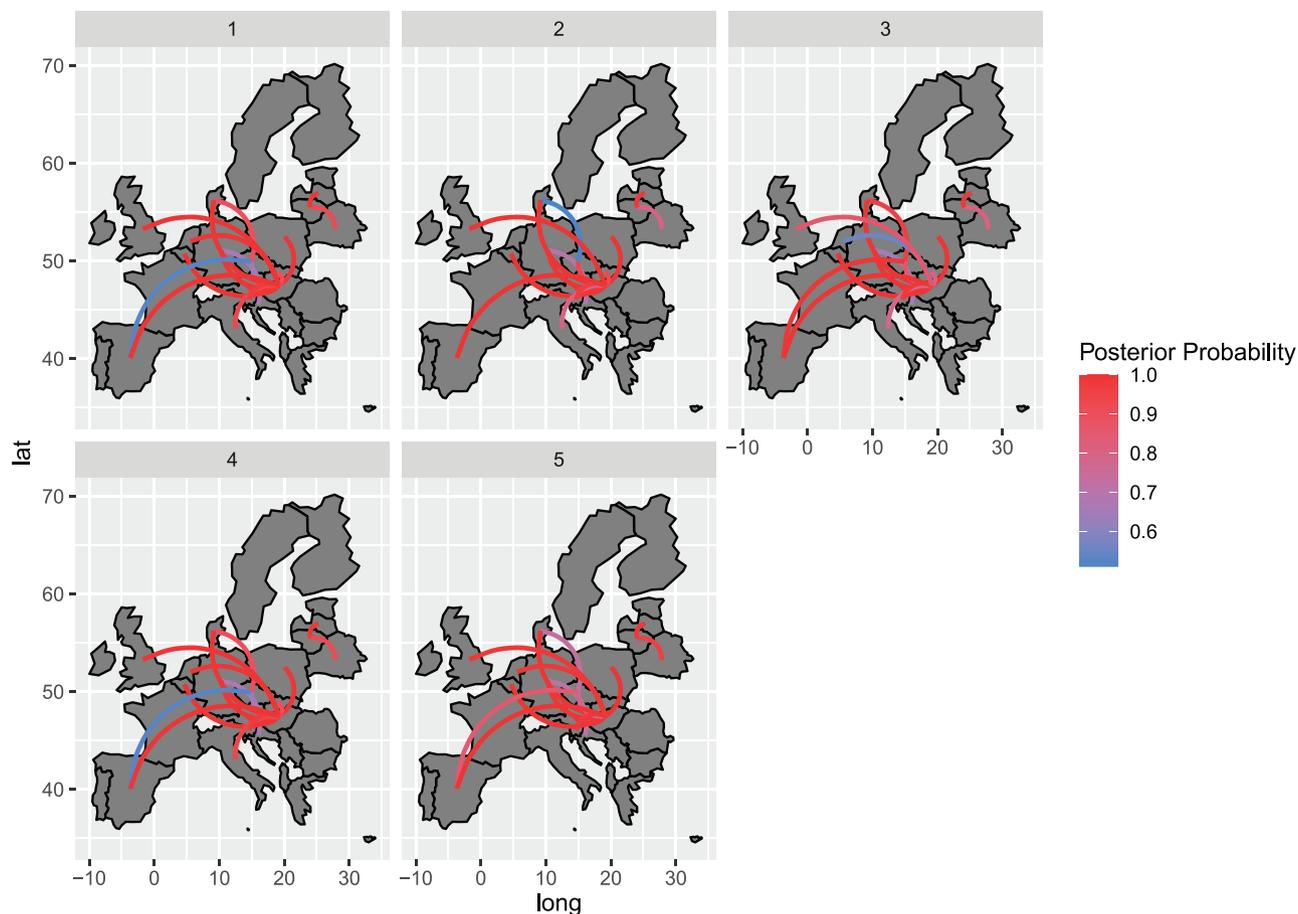
A fully overlapping scenario was observed taking into account the period after 2000, with pig trade being the only significantly associated variable (BF>100), with a positive effect size (EF = 3.34). A more variable pattern featured the previous period (i.e., before 2000), since a significant association could be identified with investments in agriculture (BF = 18; EF = −0.319), mean PRRSV strain genetic diversity (BF = 23.26; EF = 0.264) and stock density (BF>100; EF = 1.36) in the country of origin. The pig trade, although positively associated with viral dispersal (EF = 0.186), did not reach statistical significance (BF = 8.345).

FIGURE 3 Well-supported migration paths (i.e., Bayesian factor [BF]>10) among countries, estimated in the five independent BEAST runs, are depicted as edges whose colour is proportional to the posterior probability of the inferred link. The location of each country has been matched with its centroid

the consistency of the results obtained using different randomly generated data sets supports their reliability, at least when the overall pattern is considered instead of specific transmission events between country pairs. After a likely origin in Denmark, a north-to-south migration affecting Central Europe, followed by a centrifuge expansion, was observed. This pattern agrees with the one suggested by Balka et al. (2018), who hypothesized a major role of Germany in the emergence and spread of PRRSV lineage 1 (Balka et al., 2018). Thereafter, Western countries took part in a backwards spreading towards Eastern ones. On the contrary, Eastern countries acted mainly as the destination of virus importation rather than as a source. The limited commercial exchange during the Cold War likely led to the initial epidemiological isolation (Stadejek et al., 2006, 2008). In more recent years, the viral flux described herein strongly correlates with the main directionality of pig movements within Europe (Balka et al., 2018; Marquer, 2010), which, in turn, reflects the specialization of different countries in pig production (e.g., Denmark breeders, Spanish fatteners). Moreover, after the dissolution of the USSR, the pig industry in these countries decreased significantly, and this drop coincided with an increase in live pig importation from other European areas (Balka et al., 2018). Therefore, the dominant eastward PRRSV directionality, not mirrored by an opposite flow, can be justified by the political changes that occurred

over time, economically affecting the relationships between European blocks. Based on these pieces of evidence, the pig trade emerges as the likely driving force of viral spreading. The Bayesian phylogeographic GLM confirmed and provided statistical support for this hypothesis. In fact, when the overall PRRSV history was considered, the pig trade was the only predictor consistently identified as positively associated with viral dispersal, having an effect size of approximately 3 on a log scale, meaning that the viral lineage movement is orders of magnitude higher between locations with the highest pig trade compared to those without exchange. Such an effect was even more prominent when estimations were performed based on the new millennium data, when the major expansion in European trades occurred. Evidence on the relevance of animal movements in viral dispersal has been provided for other diseases of swine interest, including influenza and porcine epidemic diarrhoea (He et al., 2022; Nelson et al., 2015). Similar to the present study results, other factors made limited contributions to the spread of the infection. Additionally, the pig population size in the country of origin showed a limited impact in this sense. Likely, even though a large host population can harbour a broader viral population, the actual contribution to infection geographic expansion is essentially dependent on animal exchange. More surprisingly, in only one of the generated data sets, the low education level of farmers appeared to have a
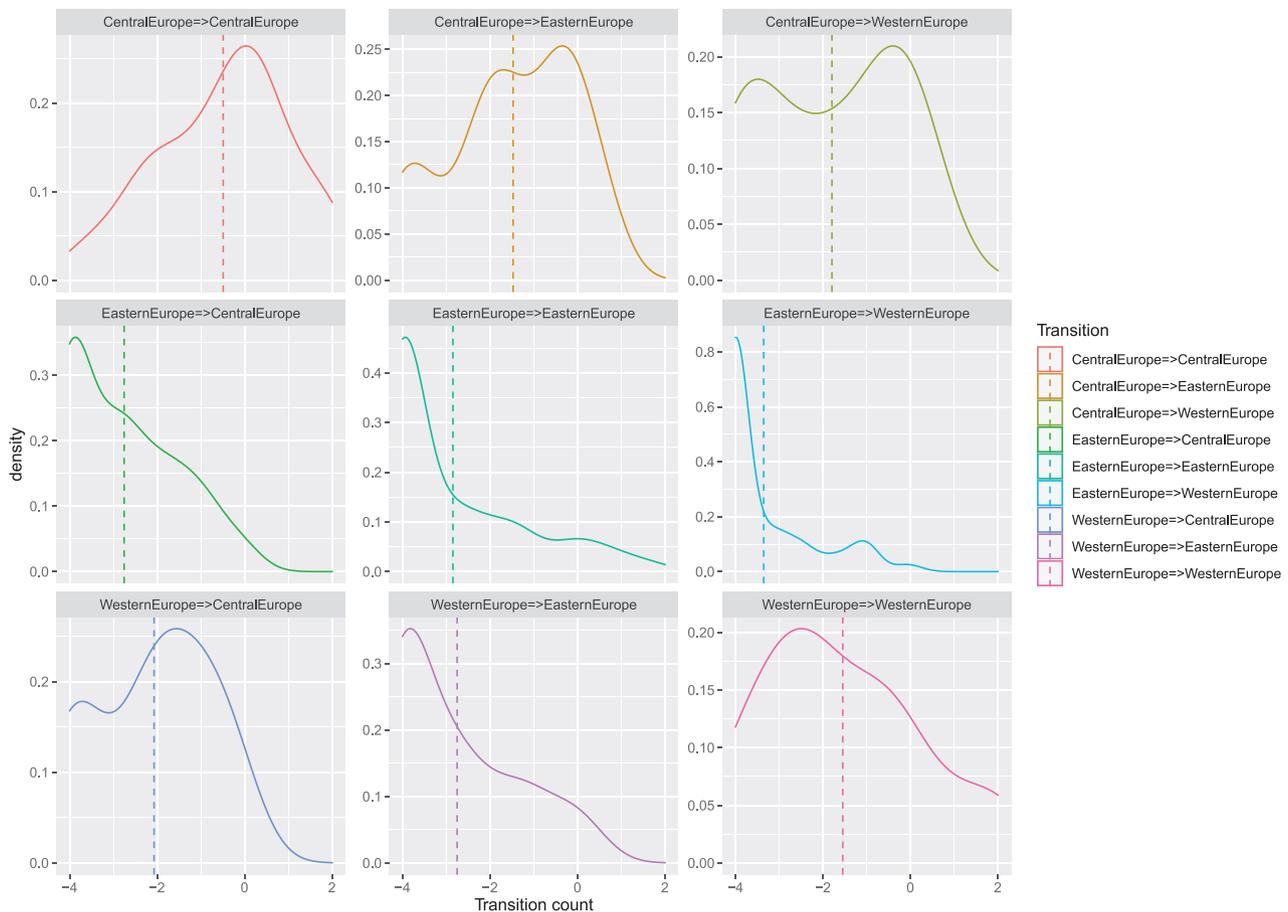
FIGURE 4 Density plot of the number of transition events (expressed as log10) among countries, aggregated by macro area. The value is indicative of the movement intensity of strains from one region to the other. The average value is shown by a dashed line

"protective effect" on viral spreading. Different studies have demonstrated the relevance of farmer and employee training in the fight against livestock infectious diseases, enhancing the implementation of effective biosecurity and control measures (Chen et al., 2021; Yoo et al., 2020). Therefore, an opposite effect would have been expected. However, it must be stressed that the overall education level does not necessarily reflect the specific knowledge and skills in animal management and infection control, thus representing a poor proxy of the studied phenomenon. Alternatively, a low education level could be correlated with poorly developed/rural farming, which displays a scarce propensity for international trade. More specific indexes representative of the actual farmers' skills would thus be necessary to further investigate and clarify this apparently paradoxical result.

Animal movements were also significantly associated with viral dispersal at the within-country level (Franzo et al., 2021), demonstrating its pivotal relevance at different epidemiological scales. Therefore, it appears clear that more resources should be directed to the controls of viral fluxes, their traceability, and the diagnosis of animal infections in asymptomatic subjects.

Interestingly, until 2000, the role of swine trade was not statistically significant based on the selected cut-off (i.e., BF>10), although other less conservative thresholds would have led to a different outcome. Nevertheless, regardless of such arbitrariness, the conditional

effect size was much lower compared to the following period, confirming the results and thus the strength of the association between swine trade and viral fluxes. On the contrary, stock density and PRRSV genetic variability had a more relevant impact on viral migration intensity before 2000. Larger animal populations have been associated with increased viral population size and thus evolutive potential, leading to genetic and phenotypic variability (Drew, 2011; Franzo et al., 2017). Such heterogeneity could provide more chances for the emergence of strains with higher transmissibility or lower cross-protection compared to strains (or vaccines) circulating in other countries, thus increasing the likelihood of the establishment of effective infections. Of note, an increase in agricultural investments revealed an inhibitory effect, meaning that adequate efforts can effectively contribute to limiting viral circulation and dispersal. The analysis of European PRRSV population dynamics largely validates this finding. A relevant decline was observed since the beginning of the new millennium, regardless of the considered randomly generated data set and irrespective of the still intense animal trade. Therefore, other factors are presumably involved in the decreased PRRSV presence. PRRSV vaccination is surely one of the most widespread and commonly applied control measures. A modified live vaccine has been available in the US since 1994, and PRRSV vaccination, with different products and protocols, has rapidly gained success thereafter and never been abandoned because of the
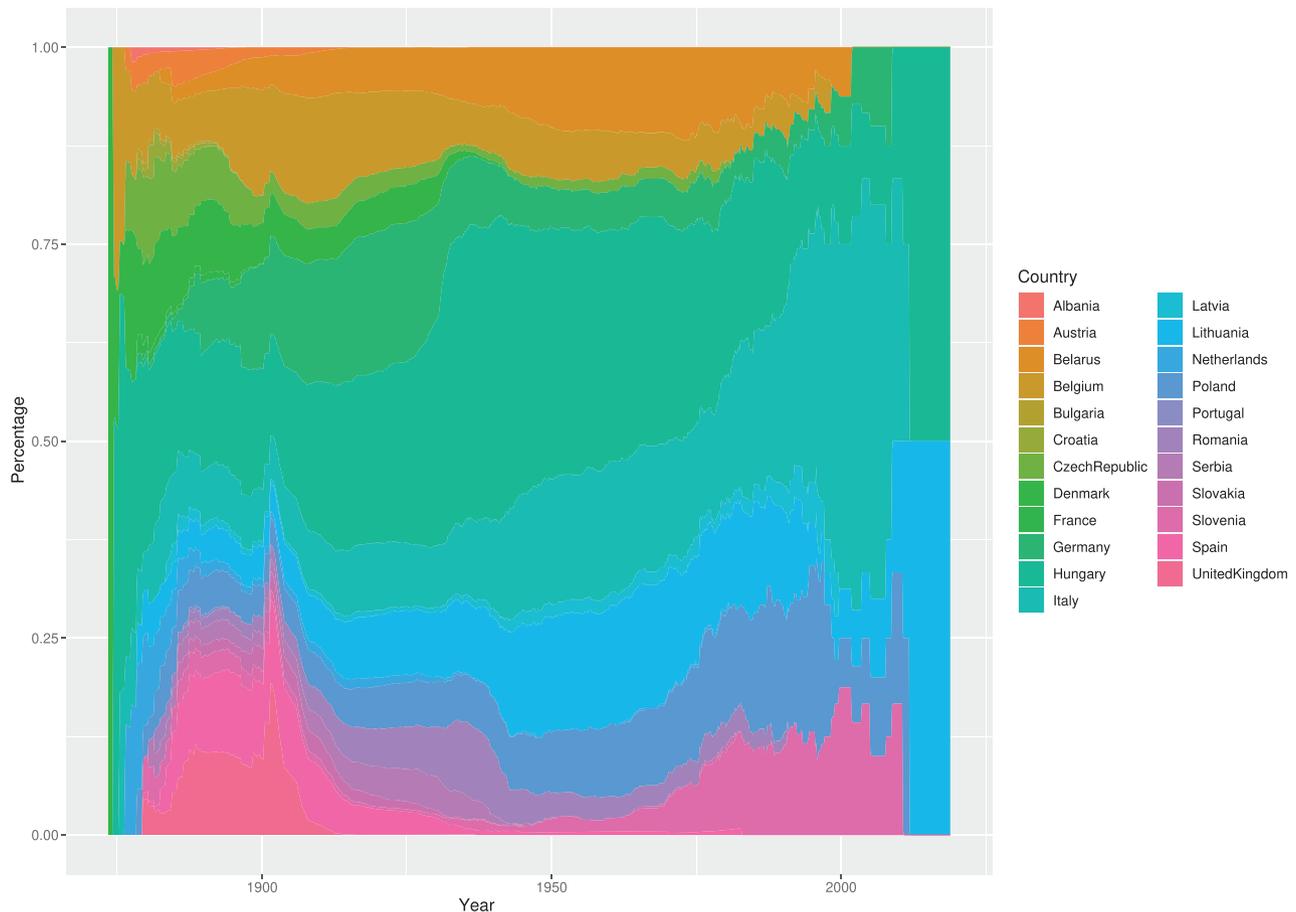
FIGURE 5 Area chart reporting the percentage of estimated lineages present in the European countries (colour-coded) over time. The width of each band is proportional to the estimated number of porcine reproductive and respiratory syndrome virus (PRRSV) lineages present in a country in a particular period

positive effects on limiting the emergence of clinical syndromes and contagiousness of the individuals (Chase-Topping et al., 2020; Fort et al., 2008; Pileri et al,. 2015; Renukaradhya et al., 2015). A relevant effect of vaccination on viral prevalence and infectious pressure can also be supported by the present study results.

On the other hand, PRRSV vaccines have been amply demonstrated to be leaky vaccines and are not fully able to prevent animal infection and viral transmission, especially in the presence of heterologous field strains (Zhou et al., 2021). Such limitations could justify some of the findings of the present study. The first is the limited effectiveness in preventing viral transmission among countries. In fact, in the presence of intense trade, as occurred after 2000, the contribution of farming investment became nonsignificant in explaining viral exchange. Such evidence reflects what was observed in Italy, where PRRSV continued to spread at high rates regardless of widespread vaccine implementation (Franzo et al., 2021).

Additionally, after a first reduction, the European viral population size tended to stabilize, confirming the incapacity not only of eradicating the infection but also of constraining viral prevalence under a certain threshold. Accordingly, the analysis of viral lineages over time demonstrated that after PRRSV introduction in a country, it continued

to circulate for the whole considered study period, although with certain fluctuations. A rational resource allocation must thus account for this limitation and evaluate alternative approaches to be included in pig health management to create multiple hurdles against PRRSV replication and transmission.

The Italian experience could be enlightening in this sense since only after the vaccination was combined with specifically designed acclimatization programs and control measures was a more effective reduction in PRRSV burden observed. Moreover, the Italian viral population dynamics, although highly similar to the European ones, were shifted by approximately 10 years (Franzo et al., 2021). Since such a scenario was reported for other swine viruses also (i.e., PCV2) (Franzo et al., 2020), an effect of the farming type and organization, largely devoted to the raising of heavy pigs for cured ham production, must be involved, hampering the implementation of effective control measures and delaying their benefits.

The results of the present study clearly demonstrate that PRRSV control must be based on an integrated approach at the UE level, benefiting from all the "weapons" available against this fastidious disease and acting at all levels of pig production, from individual farm management to intercountry trade control and coordination. This implies

the engagement of all the professional figures of the sector, as clearly stated in the 10th Manhattan principle, an old lesson whose application is evidently just at the beginning.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available in Gen-Bank at https://www.ncbi.nlm.nih.gov. These data were derived from the following resources available in the public domain: – GenBank, https://www.ncbi.nlm.nih.gov/nucleotide/.

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