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Conclusions
In the context of a naïve duck influenza virus strain, two amino acid changes away from the receptor binding site on the viral haemagglutinin could enhance growth in swine cells. Further, growth kinetics improvement in chicken cells following passaging in swine cells suggested a possible role for swine as an intermediary host for adaptation of an influenza strain from wild waterfowl to domestic chickens.

PHYLLOGENETIC ANALYSIS OF PRRSV STRAINS FROM SELECTED CENTRAL AND EASTERN EUROPEAN COUNTRIES

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Objectives
Previous phylogenetic investigations performed by co-authors of this paper revealed extreme genetic diversity in countries located at the Eastern border of Europe (Poland, Belarus, Lithuania, and Russia). Based on their findings four subtypes have been defined, and it can be concluded that strains belonging to Subtypes 2-4 can exclusively be found in those areas. In the rest of Europe, however only Subtype 1 strains have been found so far.

The aim of the study was to collect and characterize PRRSV strains from different Central and Eastern European countries, where one might expect higher degree of variation, and the possible presence of more subtypes. The main focus was set on countries that have not published detailed phylogenetic analyses about their sequences so far, namely Croatia, Romania and Serbia. Recently collected strains from Poland and Hungary were also included in the work.

Methods
Samples of serum, aborted fetuses or lungs were submitted for diagnosis to the authors’ laboratories in Poland, Hungary, Serbia, Croatia, and Romania.

Total RNA was extracted, complete ORF5 sequences were amplified and sequenced with different methods used in the laboratories. The sequences were assembled and analysed using the ClustalW software. As a reference, a set of ORF5 sequences representing the full range of EU-PRRSV diversity (all four subtypes, and sequences of MLVs) was used. The reference Type 2 sequence (VR2332), the Canadian reference strain (“Quebec”), and the American MLV sequences were also included.

Results
The sequence analyses and subsequent phylogenetic investigations revealed, that all Type 1 strains included in this study belong to Subtype 1. No Subtype 2, 3 and 4 strain was found in this study. As countries included in the study import or used to import pigs from Western EU countries (mostly The Netherlands, Denmark, and Germany) sequences similar to those countries’ sequences have also been identified. Type 1 MLV-like sequences were found in vaccinating and surprisingly in non vaccinating farms as well, suggesting the wild-type virus-like spread of those viruses. North American, MLV-like sequences we found in Croatia, Poland, and Hungary. The origins of these sequences are imported live pigs from countries that allow the use of Ingelvac MLV (Denmark, Germany). In Hungary (as reported previously) we identified non MLV-like, wild type strains, similar to the “Quebec” Canadian reference strain in two farms that belong to the same owner. The origin of that virus is unknown

Discussion
It can be concluded that Type 1 strains analysed in this study do not show the extreme degree of variability that has been observed in Belarus, Lithuania, and Russia, as they all belong to Subtype 1. These data might suggest that PRRSV got into these countries from Western European countries, where infection has been reported much earlier. In summary, the presented data confirm that Type 1 strains from countries involved in this study and Western Europe are closely related and have common origin while the strains from countries at the Eastern European borders are much more diverse. It can not be excluded however the presence of more diverse viruses in these and still non-investigated Eastern European countries. Further analyses are going to be carried out to answer this question

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