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Genomic characterization of Pseudorabies virus strains isolated from wild boar and dogs in Italy

Ana Moreno[1], Enrica Sozzi[1], Davide Lelli[1], Mario Chiari[1], Paola Prati[1], Giovanni Loris Alborali[1], Maria Beatrice Boniotti[1], Paolo Cordioli[1], Antonio Lavazza[1]

[1]*Istituto Zooprofilattico Sperimentale della Lombardia ed Emilia Romagna (IZSLER), Italy*

Pseudorabies virus (PRV) is the agent of Aujeszky's disease (AD), one of the most economically important diseases of pigs for which suids are the natural hosts. Wild boars can act as reservoirs for viral agents and may represent a potential threat for domestic animals. To deeper investigate the epidemiology of PRV in Italy, we performed the genomic characterization of one PRV strain isolated form wild boar in 2011 and 17 Italian strains originated from dogs isolated in the period 1993-2012. Out of these, ten were hunting dogs, five were working dogs living in or close to pig farms, and for the other two no data was available but they were unable to hunt. Viruses were isolated from tonsils of the wild boar and brains of dogs showing neurological symptoms by inoculation onto PK15 cell lines. Phylogenetic and molecular analysis was performed by partial sequencing of gC gene including reference and field wild boar and pig strains representatives of all the previously described clades (1, 2). Phylogenetic analysis showed that the Italian strains were divided in three clades: 1- it included three strains form working dogs closely related to pig strains isolated in Europe and America in the last 20 years, which belonged to clade B; 2 – it included ten strains from hunting dogs and the wild boar strain closely related to another wild boar Italian strain (ITA561) isolated in 1993. 3- it was formed by two working dogs and two dogs that were unable to hunt closely related to the reference strain S66 and one Brazilian strain, which were previously not included elsewhere; The clade 2 formed a separate Italian group clearly distinguishable from the clades A and B previously described (2) that included the PRV European feral strains; Clade 2 was indeed characterized by a different amino-acid deletion pattern in the gC protein (2 deletions at positions 25 and 185).

These results show a clear distinction between viral strains belonging to the first clade isolated from farm dogs and those belonging to the second clade isolated from dogs used for hunting and then traced back to the wild boar. These strains of clade 2 showed high homology to PRV strains circulating in the 70's and 80's, which today have almost disappeared in the swine population. However, the Italian group formed a clade clearly differentiate from other European wild boar strains. The third clade included four strains isolated from not hunting dogs and one isolated from an Italian pig that was not related neither to feral pig nor to recently pig strains. This study, conducted mainly on hunting dog isolates and then related to wild boars, may deepen our understanding on the epidemiology of AD in wildlife populations.

1- Fonseca et al., 2010, Vet. Microbiol. 141: 238-245

2- Muller et al., 2010, Epidemiol. Infect. 138: 1590-160

MOLECULAR CHARACTERIZATION OF ORTHOREOVIRUS ISOLATED FROM BATS, DOGS AND CATS

Davide Lelli, Ana Moreno, Alice Prosperi, Antonio Lavazza, Maria Beatrice Boniotti, Elisabetta Raffini, Paolo Cordioli

Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna – IZSLER

Orthoreoviruses, with mammalian orthoreovirus (MRV) as the type species, have been recognized as respiratory and enteric orphan viruses since 1950s. MRVs have been reported to date in various hosts, including human and animal species. In the last few years, MRVs have been often described as the sole pathogen in various hosts presenting severe clinical manifestations, such as hemorrhagic enteritis, acute respiratory infections, central nervous system implications, and others. Novel MRVs, closely related to a virus isolated in dogs in Italy in 2004, have recently been identified in bats in Italy and Germany (1,2). In addition, a novel orthoreovirus with high similarity to MRVs found in European bats was detected in Slovenia from a child with acute gastroenteritis requiring hospitalization (3). In order to obtain a clearer overview of MRVs molecular epidemiology and to verify any possibility of zoonotic transmissions, we report the molecular characterization of MRVs isolated from bats, dogs and cats in Italy.

One-hundred-ninety samples collected from bats (134 carcasses and 56 feces), 65 faecal samples collected from dogs and 31 from cats were analyzed by *in vitro* isolation using LLCMK2 cell line. A total of 25 viral strains were isolated, respectively from bats (16), dogs (6) and cats (3). They were firstly typed as MRV 3 by Multiplex RT-PCR and then genomic characterized by complete sequencing of the S1 gene (1416 bp). The sequences were compared with those of reference obtained from GenBank under alignment with the program Clustal W. The phylogenetic tree was generated by Neighbour-joining method using the Kimura 2-parameter model. Phylogenetic analysis showed that the 25 isolates were divided into 4 different clusters. The first cluster was formed by all the viruses isolated from dogs (6) and cats (3) and by 7 bat strains which showed >99% of similarity between them. The second cluster consisted of the human-MRV identified in Slovenia and two bat-MRVs detected respectively in Germany and in Italy, during this survey. The other two clusters included respectively 2 and 6 remaining Italian strains isolated from bats.

The results showed low variability among MRVs identified in dogs and cats while bat-MRVs appeared genetically more differentiated. To remark the high correlation (99.2% similarity) between the human-MRV identified in Slovenia and an Italian strain isolated from a bat (*Pipistrellus kuhlii).* In conclusion, the obtained data extend our knowledge on the epidemiology of MRVs since a limited number of nucleotide sequences are available in public database. The real epidemiological situation regarding MRVs in bats and their transmission to other animal species, including humans, should be further investigated in order to understand their full pathogenesis and zoonotic potential.

1. Lelli D *et al*., 2013. *Zoonoses Public Health*, **60**(1):84-92.
2. Kohl C *et al*., 2012. *PLoS One*, **7**(8):e43106.
3. Steyer A *et al*., 2013. *J Clin Microbiol*, **51**(11):3818-25.