

endemic in Africa, the Middle and Near East, the Indian subcontinent and China. Understanding the molecular epidemiology and evolution of PPR virus (PPRV) can assist in the control of the transboundary spread of this economically important disease. We isolated PPRV from pathological and swab samples collected 42 years apart (1969 and 2011) in Benin, West Africa, and sequenced the full genome of two isolates (Benin/B1/1969 and Benin/10/2011). Phylogenetic analysis showed that all of the characterized isolates clustered within viral lineage II and that the 2011 isolates fell into two distinct subgroups. Comparison of the full genome sequences revealed a 95.3% identity at the nucleotide level, while at the protein level, the matrix protein was the most conserved between the two viruses with an identity of 99.7% and only one amino acid substitution over the 42-year sampling period. An analysis of specific amino acid residues of known or putative function did not identify any significant changes between the two viruses. A molecular clock analysis of complete PPRV genomes revealed that the lineage II viruses sampled here arose in the early 1960s and that these viruses have likely persisted in Benin since this time.

## One-Step Multiplex RT-qPCR Assay for the detection of Peste des petits ruminants virus, Capripoxvirus, *Pasteurella multocida* and *Mycoplasma capricolum* subspecies (ssp.) *capripneumoniae*

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*PLOS One* <http://dx.doi.org/10.1371/journal.pone.0153688>

Respiratory infections, although showing common clinical symptoms like pneumonia, are caused by bacterial, viral or parasitic agents. These are often reported in sheep and goats populations and cause huge economic losses to the animal owners in developing countries. Detection of these diseases is routinely done using ELISA or microbiological methods which are being reinforced or replaced by molecular based detection methods including multiplex assays, where detection of different pathogens is carried out in a single reaction. In the present study, a one-step multiplex RT-qPCR assay was developed for simultaneous detection of Capripoxvirus (CaPV), Peste de petits ruminants virus (PPRV), *Pasteurella multocida* (PM) and *Mycoplasma capricolum* ssp. *capripneumoniae* (Mccp) in pathological samples collected from small ruminants with respiratory disease symptoms. The test performed efficiently without any cross-amplification. The multiplex PCR efficiency was 98.31%, 95.48%, 102.77% and 91.46% whereas the singleplex efficiency was 93.43%, 98.82%, 102.55% and 92.0% for CaPV, PPRV, PM and

Mccp, respectively. The correlation coefficient was greater than 0.99 for all the targets in both multiplex and singleplex. Based on cycle threshold values, intra and inter assay variability, ranged between the limits of 2%–4%, except for lower concentrations of Mccp. The detection limits at 95% confidence interval (CI) were 12, 163, 13 and 23 copies/reaction for CaPV, PPRV, PM and Mccp, respectively. The multiplex assay was able to detect CaPVs from all genotypes, PPRV from the four lineages, PM and Mccp without amplifying the other subspecies of mycoplasmas. The discriminating power of the assay was proven by accurate detection of the targeted pathogen (s) by screening 58 viral and bacterial isolates representing all four targeted pathogens. Furthermore, by screening 81 pathological samples collected from small ruminants showing respiratory disease symptoms, CaPV was detected in 17 samples, PPRV in 45, and PM in six samples. In addition, three samples showed a co-infection of PPRV and PM. Overall, the one-step multiplex RT-qPCR assay developed will be a valuable tool for rapid detection of individual and co-infections of the targeted pathogens with high specificity and sensitivity.

## Multilocus genotypic data reveal high genetic diversity and low population genetic structure of Iranian indigenous sheep

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*Animal Genetics*. 2016. doi: 10.1111/age.12429

Iranian livestock diversity is still largely unexplored, in spite of the interest in the populations historically reared in this country located near the Fertile Crescent, a major livestock domestication centre. In this investigation, the genetic diversity and differentiation of 10 Iranian indigenous fat-tailed sheep breeds were investigated using 18 microsatellite markers. Iranian breeds were found to host a high level of diversity. This conclusion is substantiated by the large number of alleles observed across loci (average 13.83, range 7–22) and by the high within-breed expected heterozygosity (average 0.75, range 0.72–0.76). Iranian sheep have a low level of genetic differentiation, as indicated by the analysis of molecular variance, which allocated a very small proportion (1.67%) of total variation to the between-population component, and by the small fixation index ( $F_{ST} = 0.02$ ). Both Bayesian clustering and principal coordinates analysis revealed the absence of a detectable genetic structure. Also, no isolation by distance was observed through comparison of genetic and geographical distances. In spite of high within-breed variation, signatures of inbreeding were detected by the FIS indices, which were positive in all and statistically significant in three breeds. Possible factors

explaining the patterns observed, such as considerable gene flow and inbreeding probably due to anthropogenic activities in the light of population management and conservation programmes are discussed.

## Identification of a new genotype of African swine fever Virus in domestic pigs from Ethiopia

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*Transbound Emerg Dis.* 2016. DOI: 10.1111/tbed.12511

African swine fever (ASF) is an important emerging transboundary animal disease (TAD), which currently has an impact on many countries in Africa, Eastern Europe, the Caucasus and the Russian Federation. The current situation in Europe shows the ability of the virus to rapidly spread, which stands to threaten the global swine industry. At present, there is no viable vaccine to minimize spread of the disease and stamping out is the main source of control. In February 2011, Ethiopia had reported its first suspected outbreaks of ASF. Genomic analyses of the collected ASF virus (ASFV) strains were undertaken using 23 tissue samples collected from domestic swine in Ethiopia from 2011 to 2014. The analysis of Ethiopian ASFVs partial p72 gene sequence showed the identification of a new genotype, genotype XXIII that shares a common ancestor with genotypes IX and X, which comprise isolates circulating in Eastern African countries and the Republic of Congo. Analysis of the p54 gene also followed the p72 pattern and the deduced amino acid sequence of the central variable region (CVR) of the B602L gene showed novel tetramer repeats not previously characterized.

## Development of broad-spectrum human monoclonal antibodies for rabies post-exposure prophylaxis

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*EMBO Molecular Medicine.* 2016. 8: 407–421. doi: 10.15252/emmm.201505986

Currently available rabies post-exposure prophylaxis (PEP) for use in humans includes equine or human rabies immunoglobulins (RIG). The replacement of RIG with an equally or more potent and safer product is strongly encouraged due to the high costs and limited availability of existing RIG. In this study, we identified two broadly neutralizing human monoclonal antibodies that represent a

valid and affordable alternative to RIG in rabies PEP. Memory B cells from four selected vaccinated donors were immortalized and monoclonal antibodies were tested for neutralizing activity and epitope specificity. Two antibodies, identified as RVC20 and RVC58 (binding to antigenic site I and III, respectively), were selected for their potency and broad-spectrum reactivity. In vitro, RVC20 and RVC58 were able to neutralize all 35 rabies virus (RABV) and 25 non-RABV lyssaviruses. They showed higher potency and breadth compared to antibodies under clinical development (namely CR57, CR4098, and RAB1) and commercially available human RIG. In vivo, the RVC20–RVC58 cocktail protected Syrian hamsters from a lethal RABV challenge and did not affect the endogenous hamster post-vaccination antibody response.

## Phylogenetic analysis of Newcastle disease viruses isolated from commercial poultry in Mozambique, 2011 to 2016

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*Virus Genes DOI: 10.1007/s11262-016-1362-6*

The complete sequence of the fusion (F) protein gene from eleven Newcastle disease viruses (NDV) isolated from commercial poultry in Mozambique between 2011 and 2016 has been generated. The F gene cleavage site motif for all eleven isolates was 112RRRKRF117 indicating that the viruses are virulent. A phylogenetic analysis using the full F gene sequence revealed that the viruses clustered within genotype VIIh and showed a higher similarity to NDVs from South Africa, China and Southeast Asia than to viruses previously described in Mozambique in 1994 to 1995 and 2005. The characterization of these new NDVs has important implications for Newcastle disease management and control in Mozambique.

## Molecular characterization of orf virus from sheep and goats in Ethiopia, 2008–2013

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*Virol J.* 2016 Feb 29;13:34. doi: 10.1186/s12985-016-0489-3

Orf is a contagious disease of sheep, goats and wild ungulates caused by orf virus (ORFV) a member of the genus Parapoxvirus, Poxviridae family. Although orf is endemic in Ethiopia, little attention has been given so far as it is not a notifiable disease by the World Organization for Animal Health. In this work, we have investigated orf