

Emerging rodent-borne viral pathogens in NE Italy: overview of seroprevalence and genomic investigations.

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Background

- Rodents play a key role as reservoirs of many zoonotic emerging infectious diseases which represent an emerging public health threat worldwide^[1;2].
- The hazard posed by rodent-borne pathogens is often underestimated since most infections are asymptomatic in the rodent hosts and underdiagnosed in human populations causing non-specific symptoms^[3].
- Rodent-borne zoonoses in Europe are mainly caused by hantaviruses and arenaviruses.

Hantaviruses: Dobrava-Belgrade virus (DOBV) Puumala virus (PUUV)

Family: Hantaviridae
Genus: Orthohantavirus
3-segmented (-) ssRNA virus
Pathology: Hemorrhagic Fever with Renal Syndrome
Mortality: <1% (PUUV); up to 15% (DOBV)
Hosts: *Myodes glareolus* (PUUV); *Apodemus* spp. (DOBV)

Arenavirus: Lymphocytic choriomeningitis virus (LCMV)

Family: Arenaviridae
Genus: Mammarenavirus
2-segmented (-) ssRNA virus
Pathology: Neurological symptoms
Mortality: <1%
Hosts: *Mus musculus*; *Apodemus* spp.



Myodes glareolus



Apodemus flavicollis



Mus musculus

Aims

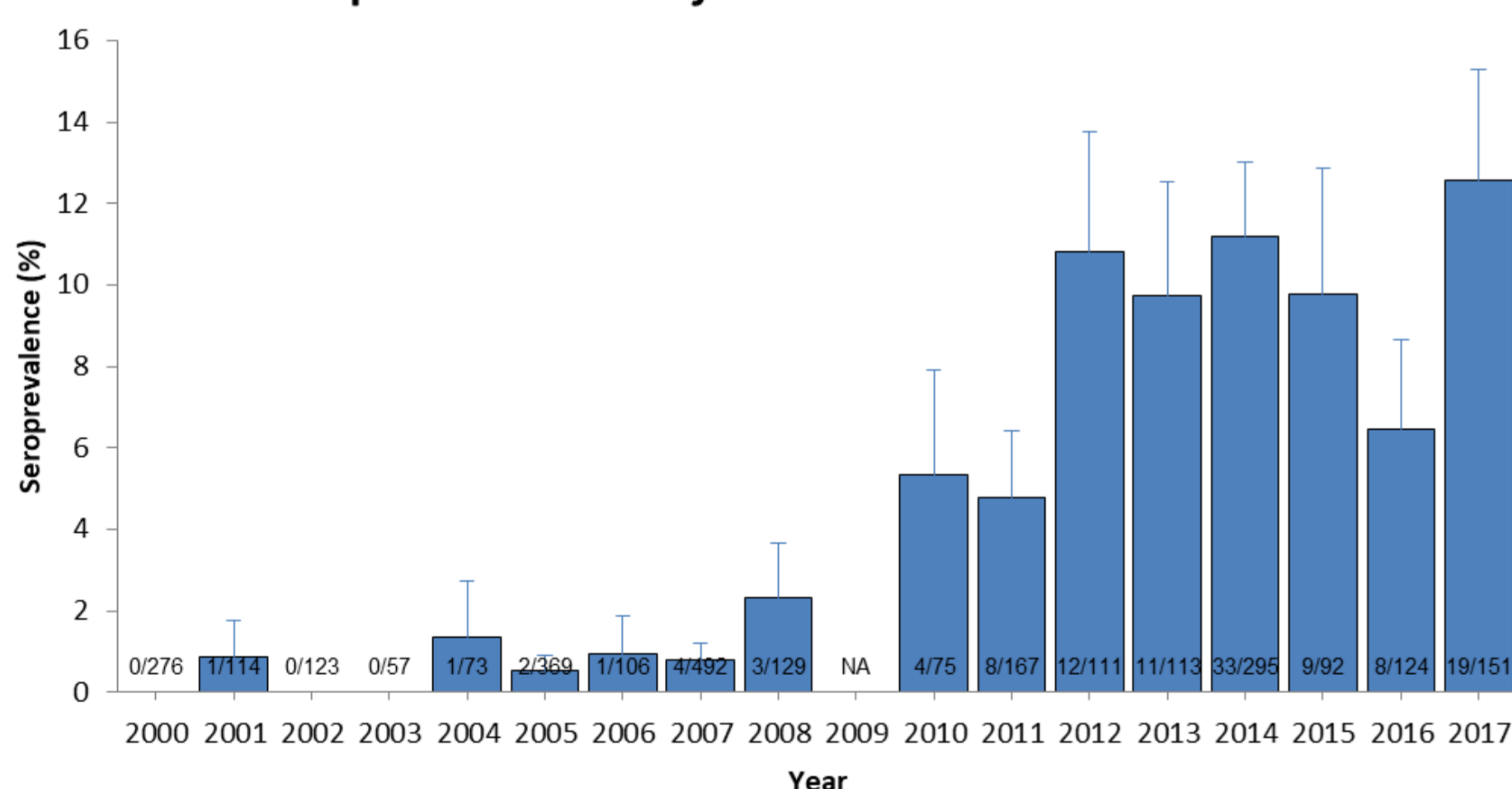
- Analyse the seroprevalence of antibodies to hantaviruses and arenaviruses in wild rodents and humans in Trentino in order to evaluate the risk of infection for humans.
- Isolate these viruses and characterize their genomes.

Results/Overview 1

SEROLOGICAL SCREENING

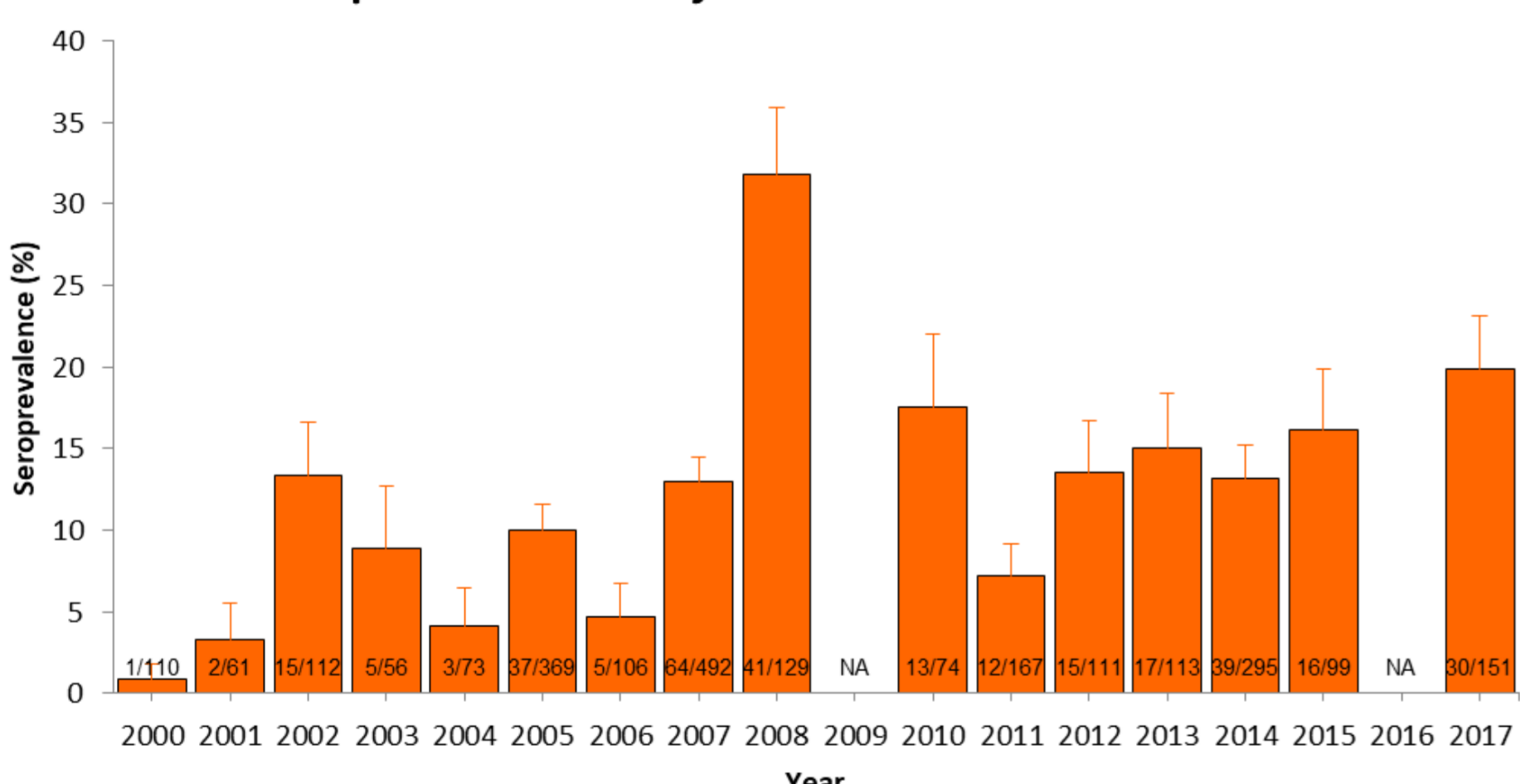
RODENTS

DOBV seroprevalence in *A. flavicollis* in Cavedine



- DOBV detected in a population of *A. flavicollis* in Trentino for the 1st time in 2001;
- recorded intermittently at low seroprevalence (~1%) until 2009
- rapid upsurge observed between 2010 and 2013, with 10.8% of the rodents found infected by 2012;
- High seroprevalence recorded till 2017 (~10%)^[7].

LCMV seroprevalence in *A. flavicollis* in Cavedine

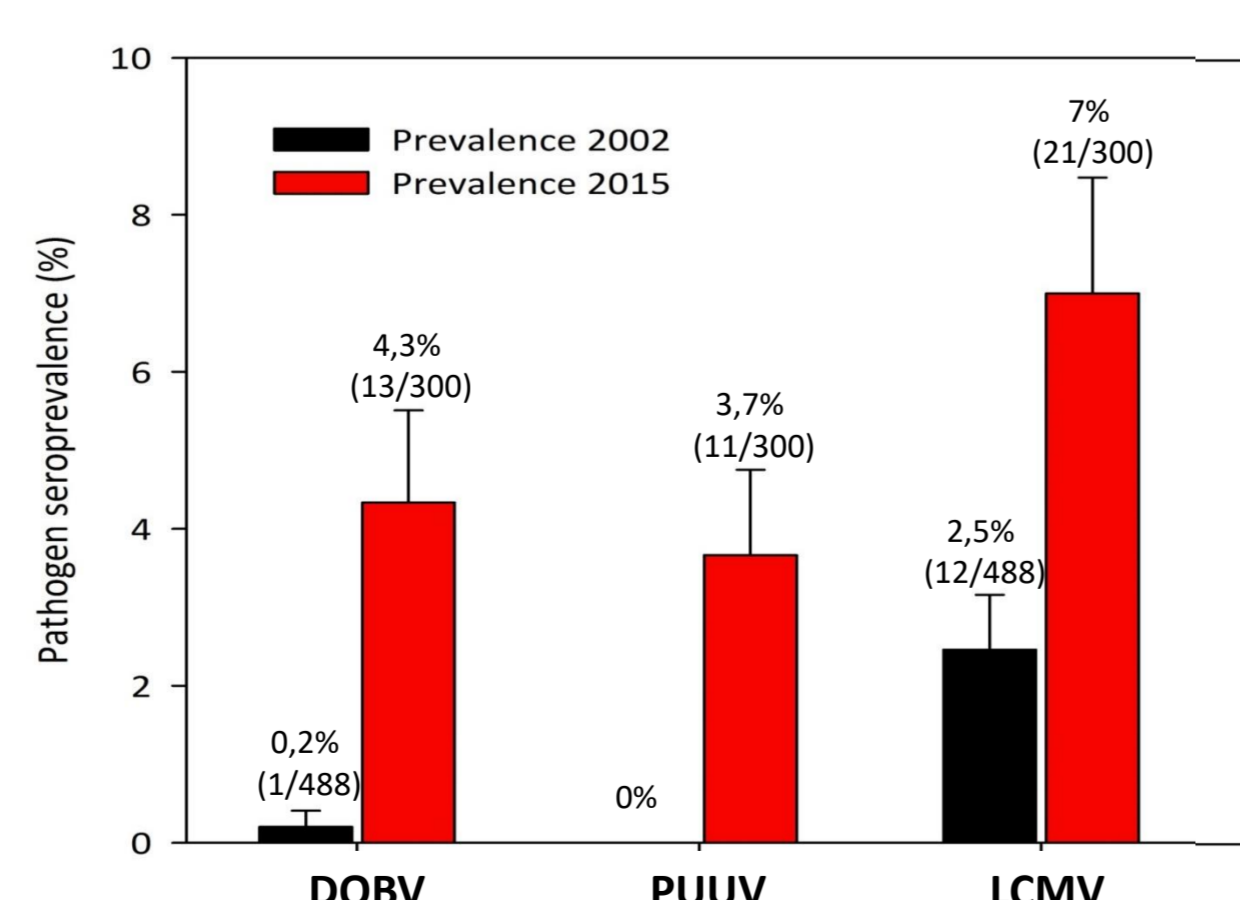


Except in 2000-2001, when it was recorded at low seroprevalence (~2%), and in 2008, when there was a peak at 31%, the seroprevalence of LCMV in *A. flavicollis* was relatively stable, with an annual mean LCMV seroprevalence of 12% (S.E. = 7.6%).

PUUV was not recorded in *M. glareolus* in Cavedine, while it was detected in the other sites at variable prevalence (0.9, 14.5, 5.6, 6.2 and 5.8% respectively in 2002, 2011, 2012, 2014 and 2015).

HUMANS

- Increase in the human seroprevalence to DOBV, PUUV and LCMV from 2002 to 2015^[8]

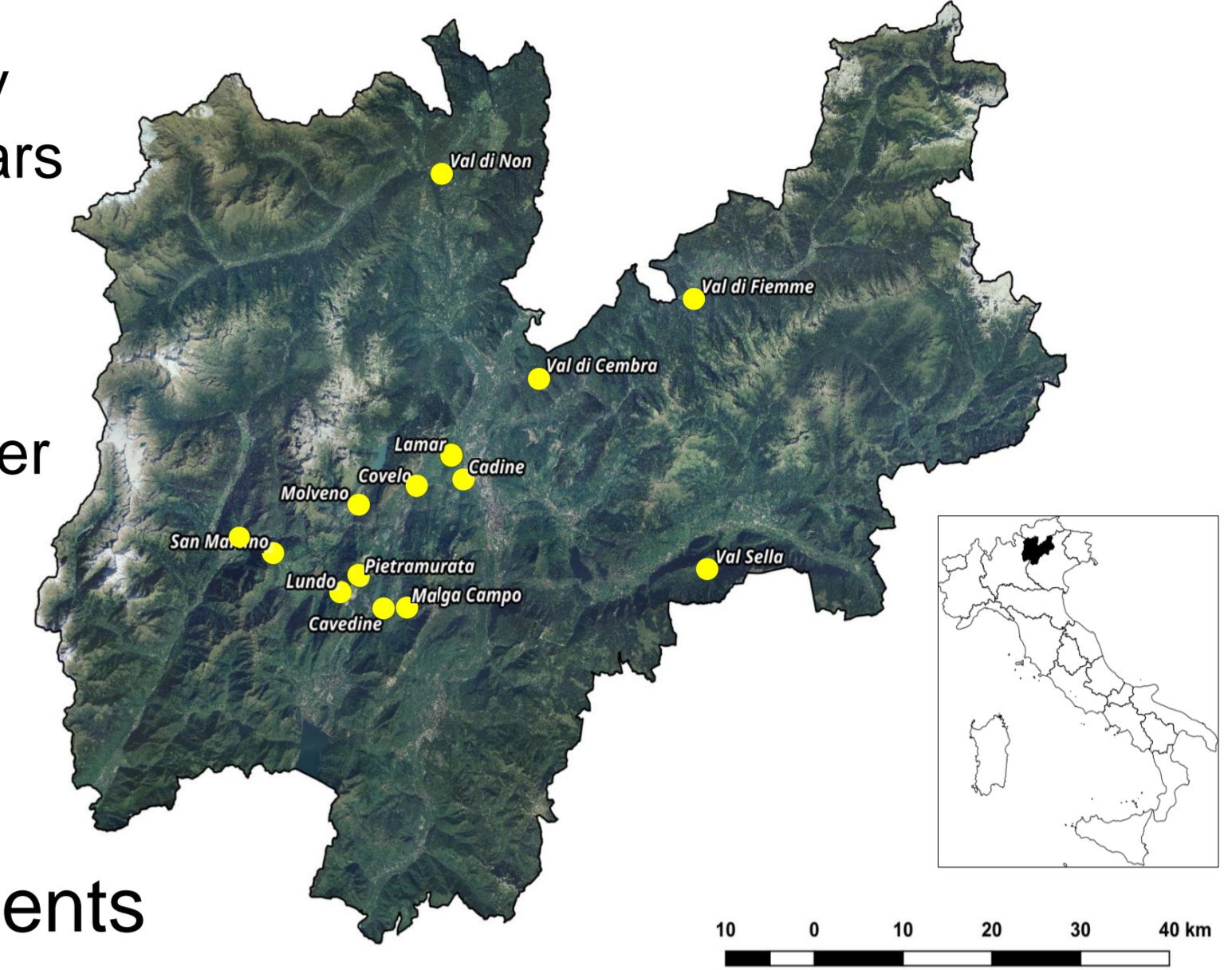


Materials & Methods

Field sampling

- Long-term live trapping (2000-2017) of rodents in Trentino (14 sites):

- One site (Cavedine) was sampled every year, while the others only in certain years
- 5613 *A. flavicollis* and 732 *M. glareolus* individuals were live-captured and pit-tagged.
- A. sylvaticus*, *Microtus* spp. and few other species were encountered rarely and not included in the analyses.
- For LCMV we only focused on *A. flavicollis* as host.



- Euthanization of seropositive rodents and dissection of target organs.

- Collection of serum samples from 488 forestry workers (high risk of rodent contact) from Trentino in 2002 and 300 in 2015.

Serological screening

Indirect immunofluorescence antibody test (IFAT) against PUUV, DOBV and LCMV IgG antibodies^[4] of 4732 *A. flavicollis*, 732 *M. glareolus* and 488 human sera.

Molecular techniques

- RT-PCR using hanta/arenavirus universal degenerate primers^[5] and specific primers;
- Targeted enrichment based on in-solution hybridization capture^[6], followed by high-throughput sequencing.

Results/Overview 2

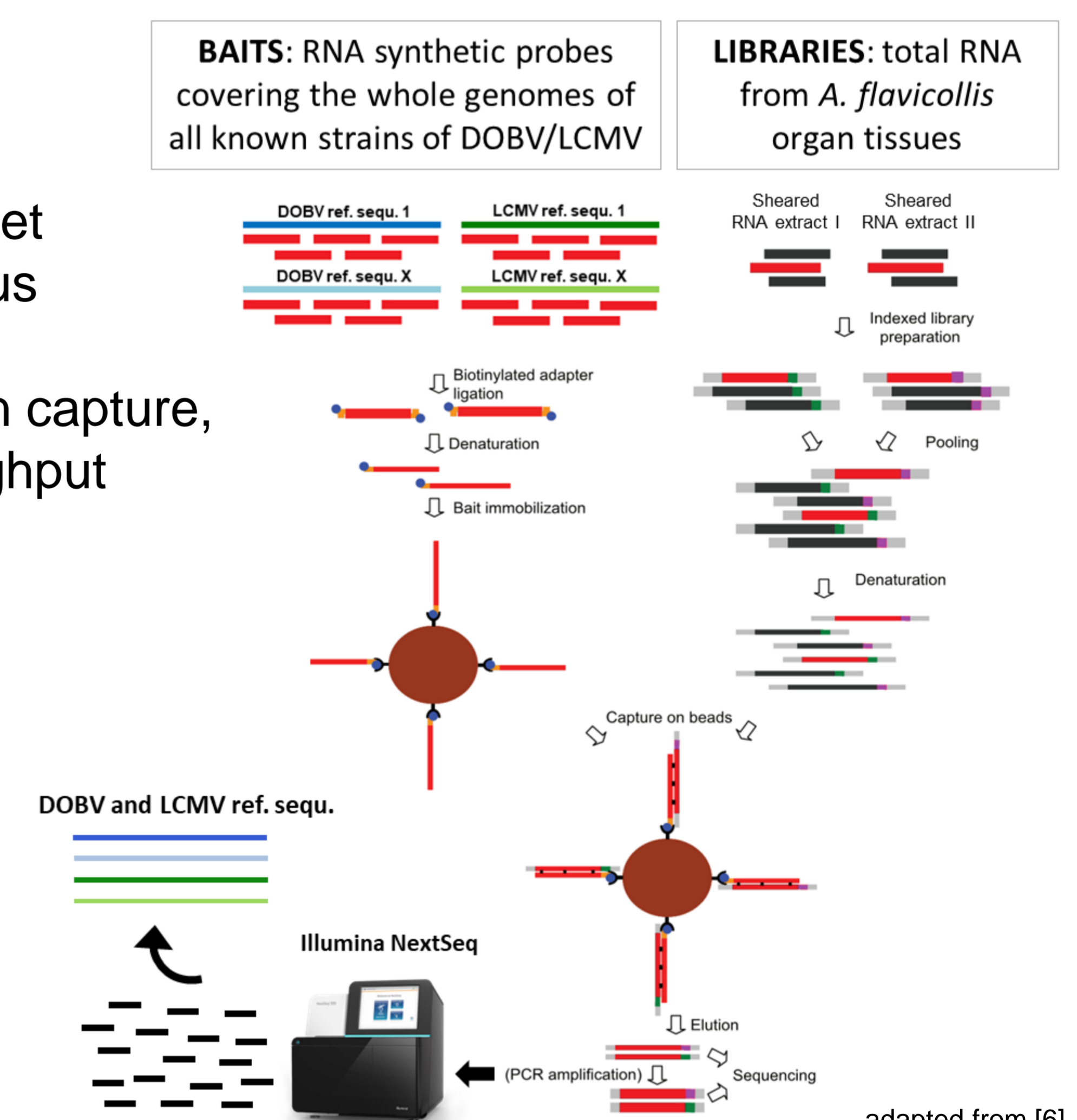
MOLECULAR IDENTIFICATION

- It has not yet been possible to detect viral RNA for DOBV and LCMV in the rodent samples by RT-PCR.

- Possible reasons:
 - very old immunity
 - very low viral load
 - infection with an as yet unidentified hantavirus

- In-solution hybridization capture, followed by high-throughput sequencing

- Advantage: overcome PCR optimization and primer mismatches issues of RT-PCR



adapted from [6]

Conclusions and perspectives

- Our long-term study confirms the presence of Hanta- and Arenaviruses in Trentino. In particular, DOBV, PUUV and LCMV have been circulating for about 15 years in the region and, even though no human cases have been reported yet, the fact that their prevalence has increased in the last decade poses concerns about a higher probability of exposure and transmission to humans.
- Particular effort should be given to warn resident people about the main risk factors to contract these infectious agents.
- The molecular identification and characterization of these pathogens will be helpful to further confirm the presence of these viruses in Trentino and understand which variants are circulating in the region and their pathogenicity.
- The finding of LCMV antibodies in another species other than *M. musculus* might indicate the existence of an as yet unknown arenavirus in Europe.

References

- [1] Bordes et al. (2015) *Integr Zool* 10:424–435. [5] Klempa et al. (2016) *Emerg. Infect. Dis* 12:838-840.
 [2] Karesh et al. (2012) *Lancet* 380:1936–1945. [6] Maricic et al. (2010) *PLoS One* 5: e14004.
 [3] Meerburg et al. (2009) *Crit Rev Microbiol* 35:221–270. [7] Rizzoli et al. (2015) *Epidemiol Infect* 134:2241–2244.
 [4] Kallio-Kokko et al. (2006) *Epidemiol Infect* 34:830–836. [8] Tagliapietra et al. (2018) *Ecohealth* doi:10.1007/s10393-018-1335-4