

## SHORT REPORT

# Recent increase in prevalence of antibodies to Dobrava-Belgrade virus (DOBV) in yellow-necked mice in northern Italy

A. RIZZOLI<sup>1</sup>†, V. TAGLIAPIETRA<sup>1\*</sup>†, R. ROSÀ<sup>1</sup>, H. C. HAUFFE<sup>1</sup>,  
G. MARINI<sup>1</sup>, L. VOUTILAINEN<sup>2,3</sup>, T. SIRONEN<sup>2</sup>, C. ROSSI<sup>1</sup>, D. ARNOLDI<sup>1</sup>  
AND H. HENTTONEN<sup>3</sup>

<sup>1</sup>Fondazione Edmund Mach – Research and Innovation Centre, San Michele all'Adige, Italy

<sup>2</sup>Department of Virology, University of Helsinki, Helsinki, Finland

<sup>3</sup>Finnish Forest Research Institute, Vantaa, Finland

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## SUMMARY

Dobrava-Belgrade virus (DOBV) is the most pathogenic hantavirus in Europe with a case-fatality rate of up to 12%. To detect changes in risk for humans, the prevalence of antibodies to DOBV has been monitored in a population of *Apodemus flavicollis* in the province of Trento (northern Italy) since 2000, and a sudden increase was observed in 2010. In the 13-year period of this study, 2077 animals were live-trapped and mean hantavirus seroprevalence was 2.7% (s.e. = 0.3%), ranging from 0% (in 2000, 2002 and 2003) to 12.5% (in 2012). Climatic (temperature and precipitation) and host (rodent population density, rodent weight and sex, and larval tick burden) variables were analysed using Generalized Linear Models and multi-model inference to select the best model. Climatic changes (mean annual precipitation and maximum temperature) and individual body mass had a positive effect on hantavirus seroprevalence. Other possible drivers affecting the observed pattern need to be studied further.

**Key words:** *Apodemus flavicollis*, Dobrava-Af seroprevalence, GLM, hantavirus, hazard.

The emergence of hantaviruses that cause haemorrhagic fever with renal syndrome (HFRS) is of particular concern for public health in Europe [1]. Moreover an increased number of HFRS cases have been recently reported in central and southern parts of the continent [2, 3]. The majority of previous studies conducted in Europe deal with Puumala hantavirus (PUUV), the most common agent of HFRS in Europe carried by the bank vole (*Myodes glareolus*); however, mice of the genus *Apodemus* host Dobrava-Belgrade virus (DOBV). DOBV-Af, or

Dobrava genotype, is a serious human pathogen associated with a case-fatality rate of up to 12%. It was first isolated from the yellow-necked mouse (*Apodemus flavicollis*) in the village of Dobrava, Slovenia [4], and subsequently reported in at least 10 European countries [1, 5–9]. Other DOBV genotypes, Kurkino and Saaremaa carried by *A. agrarius*, and Sochi carried by *A. ponticus*, have lower case-fatality rates: 0.3–0.9% for Kurkino and >6% for Sochi (values for Saaremaa not available) [5]. The nomenclature of DOBV is still under discussion [1, 5].

DOBV infections have been reported in Italy following cross-sectional seroepidemiological studies in rodents and humans since 2000 [8]. However, no clinical human cases of HFRS have been confirmed thus far [6, 8], suggesting possible underdiagnosis in

\* Author for correspondence: Mrs V. Tagliapietra, Fondazione Edmund Mach – Research and Innovation Centre, Via E. Mach 1, 38010 San Michele all'Adige (TN), Italy.  
(Email: valentina.tagliapietra@fmach.it)

† These authors contributed equally to this work.

healthcare and/or low risk due to low prevalence in rodents, unlikely to result in frequent transmission.

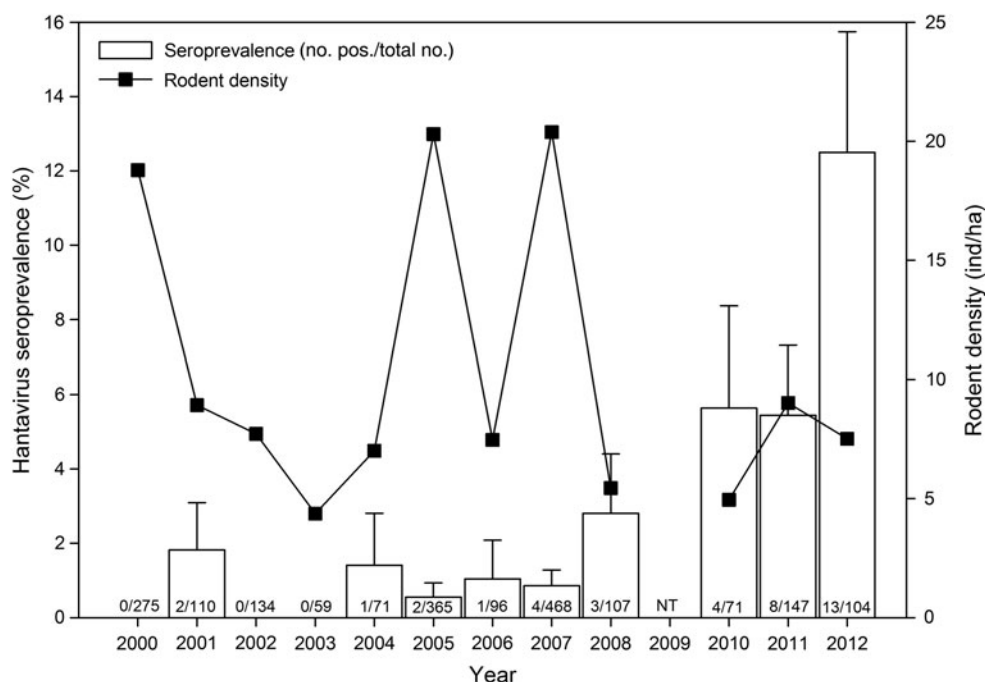
In this study, we monitored the temporal changes in risk to humans by measuring the DOBV antibody prevalence in a population of *A. flavicollis*.

Long-term research on zoonotic rodent-borne pathogens is ongoing in the province of Trento (northern Italy) in a permanent study site (municipality of Cavedine: 50° 56' 15" N, 16° 31' 13.8" E, 750 m a.s.l.), consisting of trapping grids, located on an isolated calcareous ridge dominated by a mixed deciduous broadleaf forest (*Fagus sylvaticus*, *Carpinus betulus*, *Fraxinus ornus*, *Corylus avellanae*). Trapping was performed for two consecutive nights every 2 weeks from 2000 to 2008 and every month from 2010 to present, from April to October and occasionally during winter season, for a total of 175 trapping sessions. Nine grids were monitored during 2000–2002 and four grids subsequently. Each grid has 8 × 8 (total 64) Ugglan Special live traps (model 2, Grahnb, Sweden) with a 15-m inter-trap interval. Animals were individually marked with PIT tags (Trovan<sup>®</sup> Transponders, UK, ID100) and bled once during each trapping session. Serum samples were collected and analysed using an immunofluorescence assay test (IFAT) as described by Kallio-Kokko *et al.* [8]. Tests for Dobrava were made with the Dobrava/Ano-Poroia strain, while tests for Saaremaa were made by infecting Vero E6 cells with the Saaremaa 160 V strain. Both Saaremaa and Dobrava tests were used until 2010, from when only Dobrava slides were used (since Saaremaa had never been detected). Generalized Linear Models (GLMs) with binomial error distribution were used to assess the temporal variation of hantavirus infection and to ascertain how DOBV antibody occurrence has been affected by several explanatory variables, including climatic variables (temperature and precipitation, available from 2002), rodent population density, rodent mass and sex, and larval tick burden (as a proxy for immune response). In this model, each individual was considered as appearing once in each trapping season and was considered positive if DOBV antibodies were detected at least once within the season, otherwise it was considered negative. For mice captured more than once, explanatory variables such as density, weight and larval tick burden were expressed as means across the annual trapping season. Multi-model inference was used to compare all possible models that were ranked using Akaike's Information Criterion. All variables included in the best models were ranked according

to their importance (weight), and the average coefficient for each variable was calculated. To assess temporal variation in the most important explanatory variables, GLMs with Normal and compound Poisson error distributions were implemented.

A total of 2077 individuals all belonging to *A. flavicollis* were live-captured and pit-tagged between 2000 and 2012; individuals of *A. sylvaticus*, *M. glareolus* and shrews were caught rarely, but not considered in the analysis. For the entire 13-year study period, the annual mean prevalence of antibodies to DOBV was 2.7% (s.e. = 0.3%), ranging from 0% (in 2000, 2002 and 2003; Fig. 1) to 12.5% (in 2012, 13/104; Fig. 1). Hantavirus seroprevalence from 2000 to 2012 showed a significant increase ( $t = 5.93$ ,  $P < 0.001$ ); moreover, we noted a sudden increase in prevalence of antibodies to DOBV starting in 2010 (Fig. 1). The output of the model-averaging procedure for hantavirus occurrence during the period 2002–2012 is reported in Table 1, where coefficient estimates and statistics of selected explanatory variables are also listed. With regard to climatic variables, yearly precipitation and maximum temperature both had a positive effect on DOBV antibody occurrence in mice (see Table 1). Concerning biotic factors, intra- and inter-annual fluctuation of population density, as well as sex and larval tick burden of individuals did not affect hantavirus infection levels, while rodents with higher body mass, i.e. older breeding mice, were significantly more infected. A significant increase of the mean rodent body mass was observed during the period 2000–2012 (slope =  $0.335 \pm 0.025$ ,  $t = 13.18$ ,  $P < 0.001$ ), both for breeding and non-breeding individuals. In particular breeding individuals showed a faster body mass increase (slope =  $0.345 \pm 0.03$ ) compared to non-breeding ones (slope =  $0.129 \pm 0.032$ ) ( $P < 0.001$ ).

The emergence and spillover of zoonotic pathogens, as shown for a series of disease models, are usually characterized by a lengthy, silent, latent circulation within their wildlife reservoir hosts followed by a 'jump' of the pathogen from the reservoir species to other hosts, including humans; however, the final risk of transmission depends on several regulators which often interact [10]. Therefore, monitoring the temporal dynamics of the pathogen in the reservoirs allows a very good estimation of the human risk, which is usually preceded by an increase in prevalence in the reservoir host population. Hantavirus strains are specific to their primary rodent hosts, although this infection does not appear to cause any obvious disease. In *A. flavicollis*, the virus is transmitted



**Fig. 1.** Annual prevalence of antibodies to Dobrava virus in a population of *A. flavicollis* in Trento (Italy) from 2000 to 2012. Histograms denote ratio between positive and total number of animals; vertical bar is the standard error; ■—■, mean annual density of animals per hectare; NT, no trapping performed.

**Table 1.** Importance, coefficient estimate and significance of explanatory variables remaining in the best selected models for predicting DOBV hantavirus occurrence in yellow-necked mice in Trento (Italy), from 2000 to 2012

	Importance	Coeff.	S.E.	z value	Pr(> z )
(Intercept)		-24.81	5.98	4.145	<0.001
Precipitation	1	1.08	0.28	3.823	<0.001
Temperature, maximum	1	0.92	0.32	2.881	<0.01
Weight	1	0.07	0.03	2.289	<0.05
Sex	0.56	0.87	1.20	0.721	0.47
Mouse density	0.3	-0.02	0.03	0.646	0.52
Larval tick burden	0.26	-0.002	0.01	0.151	0.88
Sex: weight	0.17	-0.04	0.06	0.656	0.51

horizontally primarily through aggressive behaviour and exposure to saliva and excreta; maternal antibodies can protect offspring from infection for some months [11]. Individuals become chronically infected, although the virus is more efficiently spread in excreta during the acute phase, associated with a high virus titres. The impact of climate change on hantavirus emergence in several countries has been recently reviewed, underlining the opposing and unpredictable effects of complex variables on its epidemiology [12]. Our results show that DOBV has circulated for at least 10 years (2000–2009) at a low prevalence within

the *A. flavicollis* study population, but starting from 2010 the prevalence suddenly increased. According to our models, variation in rodent density does not explain the observed change in seroprevalence, nor does sex or immune efficiency of individuals (as measured by larval tick burden). The increase in annual maximum temperature, although included in the model for its known positive effect on DOBV occurrence, did not significantly affect seroprevalence in the period considered. However, a rise in mean precipitation together with an increase in individual body mass were shown by our model to explain the increase in

the prevalence of antibodies to DOBV. Since such climatic changes can affect the quality and quantity of food available to rodents, the probability of survival probably also increased, resulting in a higher number of older and usually heavier animals in the population. Body mass increase, especially of breeding animals, and wetter conditions, together with higher maximum temperatures, support the hypothesis of higher survival probability and therefore prolonged viral shedding by heavier individuals, resulting in higher seroprevalence. Another option to be considered could be that changes in precipitation favoured virus survival in the environment, making transmission rate less dependent on host density [13].

At present no epidemiological data are available for the province of Trento apart from the serological study conducted on forestry workers in 2002 [8]. A new study was initiated on the basis of these results and is currently underway, but no data are currently available. This is the first detailed ecological study on DOBV, and our findings underline the importance of a closer examination of this aspect. Research on several other features of rodent population structure and behaviour is currently ongoing, e.g. the importance of variation in social structure using network analysis to transmission, as well as the pattern of exposure of the human population to the infected rodent populations.

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#### DECLARATION OF INTEREST

None

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