Seroprevalence of hantavirus infections in Switzerland in 2009: difficulties in determining prevalence in a country with low endemicity

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Citation style for this article:
Engler O, Klingström J, Aliyev E, Niederhauser C, Fontana S, Strasser M, Portmann J, Signer J, Bankoul S, Frey F, Hatz C, Stutz A, Tschaggelar A, Mütsch M. Seroprevalence of hantavirus infections in Switzerland in 2009: difficulties in determining prevalence in a country with low endemicity . Euro Surveill. 2013;18(50):pii=20660. Available online: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=20660

Article submitted on 21 December 2012 / published on 12 December 2013

In several European countries, diagnosis of nephropathia epidemica, a mild form of haemorrhagic fever with renal syndrome (HFRS) caused by Puumala virus infection, has increased over the past 10–20 years. In Switzerland, despite its geographical proximity to regions with epidemic outbreaks in Germany and France, infections are detected only sporadically. To estimate the actual prevalence and potential risk factors of human hantavirus infections in Switzerland, a seroepidemiological study was performed in 2009 on serum samples from 4,559 blood donors and 1,810 military personnel. Sera were screened using commercial Puumala IgG and hantavirus IgG enzyme-linked immunosorbent assays indicating a seroprevalence of 1% and 9%, respectively. Subsequently, the samples were analysed by immunofluorescence assay and immunoblot assay, showing a much lower prevalence, of 0.4% and 0.3%, respectively. Two of the serum samples achieved an 80% reduction in plaque-forming units in a neutralisation test. Statistical evaluation of questionnaires only identified an association of age (above 50 years) with hantavirus seropositivity when adjusted for sex (odds ratio: 2.36; 95% confidence interval: 1.10–5.05). This study provides baseline data (0.3–0.4%) for future monitoring of hantavirus seroprevalence in Switzerland and highlights the challenges in estimating the seroprevalence of these viruses in a country with very low endemicity.

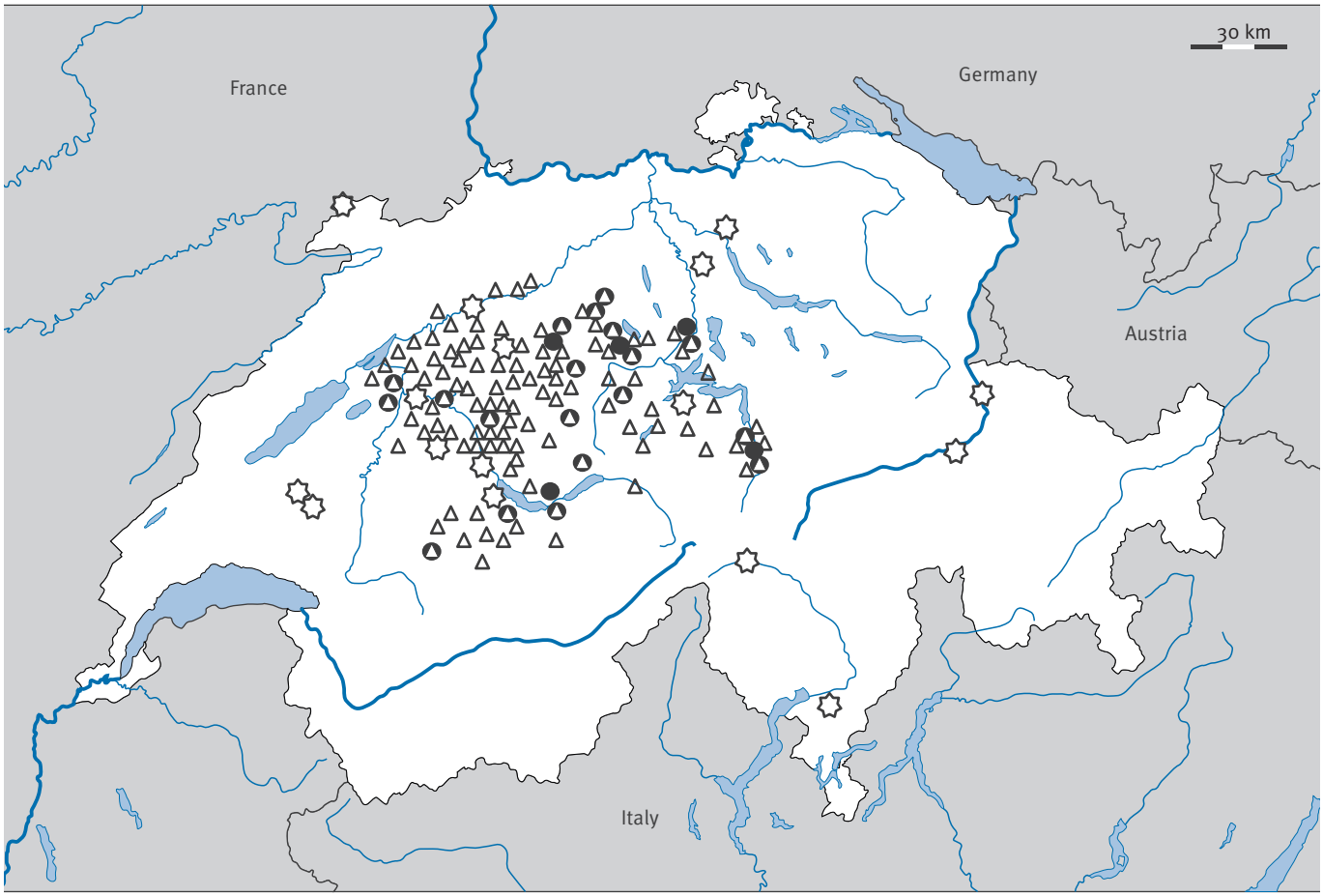
Introduction

At present, the genus *Hantavirus* includes over 20 viruses, which are mainly transmitted from rodents to humans via aerosols. Hantaviruses cause haemorrhagic fever with renal syndrome (HFRS) and hantavirus pulmonary syndrome (HPS) and are responsible for the annual hospitalisation of 150,000 to 200,000 patients worldwide [1,2]. Hantaviruses are predominantly present in distinct regions of Asia, Europe and America. In Europe, the hantavirus species Puumala virus (PUUV), Dobrava virus (DOBV) and Seoul virus (SEOV) cause HFRS in humans. These viruses differ in their geographical distribution and course of infection. PUUV is predominantly present in the European region of Russia (7,000 cases per year), Scandinavia (1,000–3,000 cases per year) and central Europe (300–3,000 cases per year) and to a lesser extent in the eastern part of Europe (Slovenia, Slovakia, Romania, Serbia) [3-5]. PUUV causes nephropathia epidemica (NE), a mild form of HFRS, which is generally not associated with major haemorrhagic symptoms and has a low case fatality rate of approximately 0.4% [6]. In the Balkan Peninsula, DOBV causes HFRS, with a case fatality rate of 9–12% [7]. Saaremaa virus (SAAV), first isolated in Estonia and found also in Russia, Slovenia and Germany, is genetically closely related to DOBV but causes a mild form of HFRS [8,9]. Although other hantaviruses have been isolated in Germany, hantavirus infections in central Europe are generally caused by PUUV [10,11].

An increase in HFRS due to PUUV infection was observed in 2005, 2007 and 2010 in distinct regions of Belgium, Luxembourg, the Netherlands, France and Germany [4]. Some of the most affected regions were located close to the Swiss border [11,12]. While Italy, which shares a border with the south of Switzerland, reported no cases of hantavirus infection between 2005 and 2010 [4], in Austria, a country neighbouring Switzerland to the east, moderate numbers of PUUV infections were reported until 2011, with an increase in the number of

Figure 1

Location of blood sampling sites and place of residence of blood donors with positive hantavirus serology, Switzerland, 2009



Blood samples were collected from 16 military bases throughout Switzerland by a field team of the University of Zurich/ Institute of Social and Preventive Medicine, Division of Communicable Diseases and from 114 locations by mobile teams of the Blood Transfusion Service of the Swiss Red Cross Berne.

Place of residence of blood donors with positive hantavirus serology (positive enzyme-linked immunofluorescence assay screening followed by either a positive immunofluorescence assay and/or a positive or questionable immunoblot assay) is indicated. Dark-grey lines indicate borders; rivers and lakes are depicted in blue.

Map adapted from: http://d-maps.com/carte.php?num_car=2648&lang=en

human cases in 2012 in provinces bordering Slovenia [13]. Despite the proximity of Switzerland to endemic regions in Germany and France, only one case was reported here between 1988 and 2003 (Nicole Gysin, Federal Office of Public Health, personnal communication, 11 December 2013) and a few HFRS patients (between 0 and 4 cases per year) were documented in Switzerland between 2003 and 2011 [14]. However, as the majority (90–95%) of PUUV infections remain subclinical [15] and symptomatic infections may easily be overlooked, due to lack of awareness among Swiss clinicians, the actual number of hantavirus infections may be underestimated in Switzerland.

IgG antibodies produced in response to hantavirus infection persist for 20 years or more [16]. Hence, serological studies can be used to determine the proportion of a population that has been infected with hantaviruses. In European countries where infections with hantaviruses are common, the IgG seroprevalence ranges from 1% to 9% [1-3]. In Germany, the average seroprevalence was estimated at 1–2% in 1995, but

was much higher in 2005 in epidemic areas such as Baden Württemberg and Lower Bavaria (about 7%) [17-19]. A regional and smaller serological study performed in the north-eastern part of Switzerland in 2002–03 indicated that the hantavirus seroprevalence in local blood donors was in the range of 0.5%, with comparable results in selected risk groups such as forestry workers and farmers [20]. A higher seroprevalence was observed among young soldiers tested during their military service (1.9%; 2/103), but the sample size was small and the difference was not statistically significant [20].

Our study aims were threefold: firstly, to estimate the actual PUUV seroprevalence in blood donors in central Switzerland, to provide baseline data for surveillance; secondly, to determine whether there would be a statistically significant difference between the seroprevalence in army personnel and blood donors when the sample size is larger; and thirdly, to generate evidence to increase awareness and preparedness given the cyclical epidemic situations in our neighbouring countries.

We performed a sample size determination based on previous seroprevalence data for Switzerland [20] and Swiss military personnel and blood donors were selected as study populations. Since it is difficult to assess the seroprevalence in a country with low endemicity, we combined the high sensitivity of two ELISAs used for screening with the specificity provided by immunofluorescence, immunoblot and neutralisation assays to confirm the positive sera.

Methods

Study population, data collection and selection criteria

During 2009, a prospective questionnaire-based seroprevalence study was performed in Switzerland. The study protocol was approved by the relevant cantonal ethical boards. To be enrolled, adults (>18 years) had to be German-, French- or Italian-speaking Swiss residents and either soldiers of the Swiss Armed Forces during their military service or registered blood donors.

We selected 16 military bases on the basis of their location throughout Switzerland. All soldiers at the bases, who resided all over the country, were invited to participate in the study. Participation was voluntary. All soldiers were informed orally about the study, asked to give their written consent and to complete a structured questionnaire on the military base before they provided a single blood sample.

All blood donors registered with mobile teams of the Blood Transfusion Service of the Swiss Red Cross Berne received information about the study, the questionnaire and the consent form by post and were asked to take the completed forms to their next blood-donation session, if willing to participate. Samples and forms from registered blood donors were collected in 114 locations within the cantons of Berne and Lucerne and in central Switzerland (Figure 1). Sera were taken during the regular blood donation organised by the mobile teams and uncertainties concerning the questionnaire or study were clarified on site. Testing blood samples obtained by the mobile teams ensured that donors living in rural areas were included, as the teams visit villages outside urban areas.

Potential risk and confounding factors, such as place and location of residence, demographics, occupational and leisure activities, self-perceived current health status, relevant symptoms, smoking history, comorbidities and travel history in the past two years were assessed with the questionnaire.

Serological screening

All sera were screened for hantavirus-specific IgG using commercially available ELISAs. Sera were first analysed with the Hantavirus IgG DxSelect ELISA (Focus, Cypress, USA) then with Hantavirus Puumala IgG/IgM ELISA (Progen Biotechnical, Heidelberg, Germany).

Immunofluorescence assay

Serum samples that tested positive with at least one of the ELISA tests were further analysed by immunofluorescence assay (IFA) using the Euroimmun Anti-Hantavirus-IIFT Mosaic II Test (Euroimmun, Lübeck, Germany). Briefly, 1:100 diluted serum samples were added to each reaction field on biochips containing either uninfected cells or cells infected with PUUV, SAAV, DOBV, Hantaan virus (HTNV) or SEOV.

Immunoblot assay

All ELISA-positive sera were further analysed using the recomLine Bunyavirus IgG/IgM test kit (Mikrogen, Neuried, Germany). In short, serum samples diluted 1:100 were incubated on recomLine test strips containing six lines with complete nucleocapsid proteins from HTNV and PUUV, or a recombinant N-terminus of the nucleocapsid antigen from PUUV, HTNV, DOBV, SEOV or from sandfly fever virus serotype Toscana (TOSV), as well as a control band for the antibody class (IgG or IgM).

Focus reduction assay

The focus-reduction neutralisation test (FRNT) was performed as described previously [21]. An 80% reduction in the number of focus forming units (FFU) compared with the virus control was used as the criterion for virus neutralisation titres.

Sample size determination and statistical analysis

The sample size determination was based on previous data from Switzerland [20] and consisted of a two-sample comparison of proportions with a ratio of 0.5 between military personnel and blood donors (power 80%, two-sided, p=0.05). For the statistical analysis, a positive ELISA combined with either a positive IFA

Table 1

Prevalence of hantavirus antibodies in the study populations by serological tests, Switzerland, 2009 (n=6,369)

Source of sera	Number of sera tested	Number (%) of sera found positive or borderline for hantavirus antibodies			
		Hantavirus ELISA	PUUV ELISA	IFA	IBA
Blood donors	4,559	405 (8.9)	40 (0.9)	22 (0.5)	13 (0.3)
Military personnel	1,810	194 (10.7)	19 (1.0)	4 (0.2)	3 (0.2)
Total	**6,369**	**599 (9.4)**	**59 (0.9)**	**26 (0.4)**	**16 (0.3)**

ELISA: enzyme-linked immunosorbent assay; IBA: immunoblot assay; IFA: immunofluorescence assay.

result and/or a positive or questionable immunoblot assay (IBA) was classified as a positive hantavirus serology (denoting a case).

Due to the small number of cases, our analysis was restricted to the main potential risk factors. All variables were assessed univariately and by subsequent stepwise backward logistic regression using positive hantavirus infection as the outcome (STATA version 12.1).

Results

Study population

A total of 1,810 blood samples were collected from military personnel from May to December 2009. The participation rate was 49.3% (1,810/3,673), of which 1,797 (99.3%) were male and 13 (0.7%) were female. The ages of the participating personnel ranged from 18 to 56 years, with a median of 21 years.

A total of 4,559 samples were collected from blood donors in 114 locations during July to November 2009 (Figure 1). The participation rate was 48.7 % (4,559/9,359), similar to that of the military personnel; however, men (2,720; 59.7%) and women (1,743; 38.2%) were more equally represented. Data on age were unavailable for 96 (2.1%) of the donors sampled; the median age of the rest was 45 years (range: 18–65 years).

Screening by enzyme-linked immunofluorescence assay

All sera were screened for hantavirus IgG using two different ELISA test systems. When the hantavirus ELISA was used, 405/4,559 serum samples from blood donors and 194/1,810 samples from military personnel were positive, corresponding to a seroprevalence of 8.9% and 10.7%, respectively (Table 1). However, according to the results obtained with the PUUV ELISA, 40 samples from the blood donors and 19 samples from the military personnel gave positive results, corresponding to a prevalence of 0.9% in the blood donors and 1% among military personnel.

Analysis by Immunofluorescence and Immunoblot assay

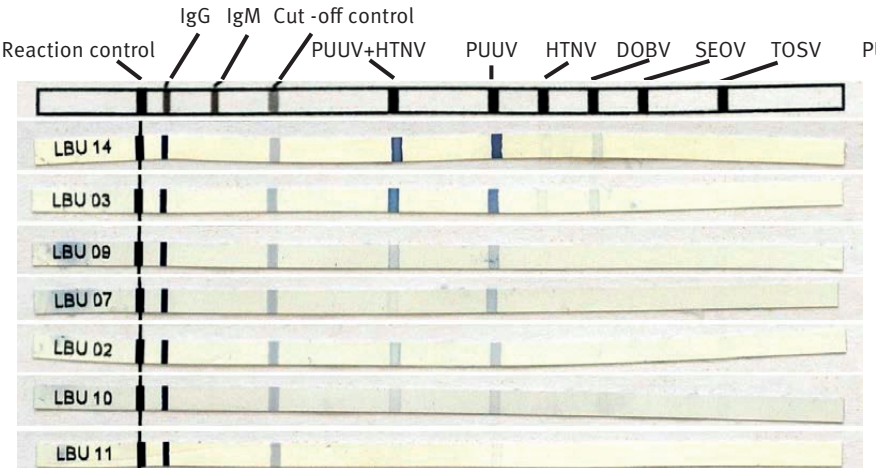
To obtain reliable seroprevalence data, following broad screening using two different ELISAs, all positive samples from both ELISAs (n=655) were further analysed by IFA and IBA. Both tests allow us to differentiate between hantaviruses causing disease in Europe and were used to confirm the ELISA results. Of the 655 serum samples tested by IFA, 25 showed positive results for PUUV; six of these sera produced a strong or very strong immunofluorescence signal and 19 sera showed a weaker but still specific PUUV-fluorescence pattern (data not shown). Cross-reactivity with other hantaviruses (HTNV, SEOV, DOBV, SAAV) was observed for several sera (n=11), but differentiation was possible in most cases (n=8) by comparing the signal intensities to the different hantaviruses. One serum showed a specific reaction for the SAAV or DOBV antigen) and two sera showed a comparable fluorescence signal for PUUV and SAAV and/or DOBV.

When analysed by IBA, even fewer (n=16) of the 655 sera tested showed positive or questionable band patterns for PUUV or other hantaviruses (illustrated in Figure 2). In most sera (11/16), the signal was specific for PUUV with only minimal cross reactivity to the antigens of other hantaviruses (Table 2). Two sera reacted specifically to DOBV, with minimal cross reactivity; another showed a strong signal for both DOBV and HTNV. Several sera (n=24/6,369) reacted against the TOSV antigen (data not shown). The agreement between the different tests was good for sera producing strong IFA and IBA results but was only around 50% when the immunofluorescence signal was weak (Table 2).

In summary, of all 655 ELISA-positive sera analysed in more detail, 30 showed clearly positive results by IFA and/or IBA. A total of 26 were positive by IFA and 16 gave positive (n=9) or questionable (n=7) band pattern in the IBA, resulting in an overall seroprevalence of the 6,369 sera tested of 0.4% (IFA) and 0.3% (IBA). The seroprevalence in the blood donors (4,559 sera) was 0.5% when confirmation was based on the IFA and 0.3% when IBA was used.

Figure 2

Immunoblot pattern of serum samples analysed to confirm IgG antibodies against hantaviruses, Switzerland, 2009



-	no band
+/-	weak band
+	band of equal intensity as cut-off
++	band of stronger intensity than cut-off

IBA: immunoblot assay; DOBV: Dobrava virus; HTNV: Hantaan virus; neg: negative; pos: positive; PUUV: Puumala virus; quest: questionable; SEOV: Seoul virus.

All sera (n=655) from blood donors and military personnel with a positive ELISA were confirmed by IBA. The immunoblots show examples of sera from blood donors rated positive, questionable and negative according to the band intensities. Serum samples were rated positive, if the PUUV+HTNV band was of stronger intensity compared with the cut-off-control (examples 4476 and 682) or if of equal intensity; at least one additional band of hantavirus serotype PUUV, HTNV, DOBV or SEOV had to be of at least equal intensity (example 2076). The result was estimated as questionable if only one of the bands was of equal intensity as the cut-off band (examples 183 and 1387) and negative, if the signal was weaker than the cut-off (example 2270) or absent (example 2798).

Confirmation by focus reduction neutralisation test (FRNT)

Of the 30 sera showing clearly positive results by IFA and/or IBA, 14 were analysed for their neutralising capacity against PUUV and HTNV. At a dilution of 1:40, only two sera (numbers 682 and 4476) could neutralise these hantaviruses (Table 2). While serum 624 was also capable of neutralising PUUV at a serum dilution of 1:80, serum 4476 was not. Both sera showed very strong positive results in the IFA and IBA assays. The other two sera with comparably strong reaction patterns for PUUV by IFA and IBA (numbers 149 and 3051) did not reach the 80% reduction level required for a positive result in the FRNT. Nevertheless, they achieved a reduction of greater than 50% in FFU, indicating that neutralising antibodies against hantaviruses might be present. Sera with only weak signals in the IFA or IBA did not lead to a significant reduction of FFU in the FRNT (data not shown).

Evaluation of risk factors for infection

To assess potential risk factors for hantavirus infection, univariate and multiple backwards logistic regression analyses were performed on positive serum samples cases (defined as having a positive ELISA test and confirmatory IFA and/or positive or questionable IBA results). No significant association of risk factors, such as recreational activities or travel to endemic countries with seropositivity was identified: only age (above 50 years) was associated with an increased risk of seropositive ELISA and IFA or IBA assay. When dichotomised into two age groups (≤50, >50 years) and controlled for sex, the odds ratio was 2.36 (95% confidence interval (CI): 1.10–5.05), which indicates an approximately 2.5-fold increased risk of hantavirus infection for people over 50 years-old. By place of residence (postal code) of 24 of the 25 blood donors with positive serum samples (information on postal code was missing for one donor), no clustering could be detected (Figure 1).

Discussion

The seroprevalence of hantavirus infections of 0.3–0.5%, estimated in blood donors from central Switzerland, is low compared with the seroprevalence observed in endemic regions of surrounding countries such as Baden-Württemberg in south-west Germany (about 2–3% [17,19]) but seems to be somewhat higher than the prevalence found in other countries where no

Table 2

Summary of serological data of hantavirus-reactive serum samples, Switzerland, 2009 (n=30)

Serum ID	Hantavirus ELISA		PUUV ELISA		IFA						IBA						FRNT	
						Signal intensity						Band intensity						
	Result	OD S/Cal	Result	OD S/Cal	Result	HTNV	PUUV	SEOV	SAAV	DOBV	Result	PUUV/ HTNV	PUUV	HTNV	DOBV	SEOV	PUUV	HTNV
Blood donors																		
149	pos	4.474	eqv	1.373	pos	+	++	-	-	-	pos	++	++	-	-	-	neg	neg
183	neg	0.707	pos	1.655	pos	-	+	-	-	-	pos	+	+	-	-	-	neg	neg
682	pos	1.614	eqv	1.412	pos	+	+++	-	+	+	pos	++	++	+/-	+/-	-	1:80	1:40
977	pos	1.326	neg	0.355	neg	-	-	-	-	-	pos	++	+/-	+/-	++	-	ND	ND
990	neg	0.171	pos	2.272	pos	-	++	-	-	-	neg	-	-	-	-	-	ND	ND
1064	pos	1.742	neg	0.243	pos	+	+	+	+	+	pos	++	-	++	++	-	ND	ND
1387	pos	1.337	eqv	1.285	neg	-	-	-	-	-	quest	-	+	-	-	-	neg	neg
1474	pos	1.611	neg	0.295	pos	-	+	-	-	-	neg	-	-	-	-	-	ND	ND
1483	neg	0.480	pos	1.585	pos	-	+	-	-	-	quest	-	+	-	-	-	neg	neg
2076	neg	0.845	pos	1.752	pos	+	+	-	-	-	pos	+	+	-	-	-	neg	neg
2270	neg	0.743	pos	1.633	pos	+	+	-	-	-	neg	+/-	+/-	-	-	+/-	neg	neg
2404	neg	0.242	pos	1.656	pos	-	+	-	-	-	quest	+	-	-	-	-	ND	ND
2511	pos	1.847	neg	0.185	pos	-	+	-	-	-	neg	-	-	-	-	-	ND	ND
2551	pos	2.047	neg	0.188	pos	-	+	-	-	-	neg	-	-	-	-	-	ND	ND
2798	neg	0.092	pos	2.893	pos	-	+	-	-	-	neg	-	+/-	-	-	-	neg	neg
3051	pos	2.187	eqv	1.081	pos	+	++	-	-	-	pos	++	++	-	-	-	neg	neg
3115	pos	3.115	neg	0.297	pos	-	+	-	+	+	neg	-	-	-	-	-	ND	ND
3389	pos	1.205	neg	0.472	pos	-	+	-	-	-	quest	+/-	+	-	-	-	neg	neg
3529	pos	1.889	neg	0.351	pos	-	+	+	+	-	neg	-	-	-	-	-	ND	ND
3585	neg	0.245	pos	1.628	pos	-	+	-	-	-	neg	-	-	-	-	-	ND	ND
4162	pos	3.487	neg	0.382	pos	-	+	-	-	-	neg	-	-	-	-	-	ND	ND
4304	neg	0.307	pos	1.917	pos	-	+	+/-	-	+/-	neg	-	-	-	-	-	ND	ND
4320	pos	3.707	neg	0.311	pos	-	+	-	-	-	neg	-	-	-	-	-	ND	ND
4476	pos	3.421	pos	1.741	pos	+	+++	+	++	++	pos	++	++	+/-	+	-	1:40	1:40
4521	pos	3.024	pos	1.589	neg	unsp	unsp	unsp	unsp	unsp	quest	+/-	+	-	-	-	neg	neg
Military personnel																		
I 147	pos	1.578	neg	0.557	neg	-	-	-	-	-	pos	++	-	-	-	-	ND	ND
L55	pos	4.012	pos	1.644	pos	-	+	-	-	-	quest	+/-	+	-	-	-	neg	neg
R1	pos	3.911	neg	0.351	pos	-	-	+/-	+	+	quest	-	-	-	+	-	ND	ND
M14	neg	0.153	pos	1.807	pos	-	+	-	-	-	neg	-	-	-	-	-	ND	ND
X6	neg	0.659	pos	2.164	pos	-	+++	-	-	-	neg	+/-	+/-	-	-	-	neg	neg

Cal: calibrator; DOBV: Dobrava virus; ELISA: enzyme-linked immunosorbent assay; eqv: equivocal; FRNT: focus reduction neutralisation test; HTNV: Hantaan virus; IFA: immunofluorescence assay; ND: not done; neg: negative; OD: optical density; pos: positive; PUUV/HTNV: Puumala or Hantaan virus; PUUV: Puumala virus; ques: questionable; S: sample; SAAV: Saaremaa virus; SEOV: Seoul virus nucleocapsid protein; unsp: unspecific.

IFA

-	no signal
+/-	barely visible signal
+	weakly positive signal
++	clearly positive signal
+++	bright, positive signal

IBA

-	no band
+/-	weak band
+	band of equal intensity as cut-off
+ +	band of stronger intensity as cut-off

Listed are all samples that were found positive by ELISA screening and could be confirmed by either IFA or IBA. The OD ratio (OD of the sample divided by the OD of the calibrator for the two ELISAs and the signal intensities observed by IFA and IBA are indicated. Sera showing a positive signal by IFA or IBA were further analysed by FRNT. When positive, the serum dilution at which 80% focus reduction was achieved is indicated.

or only very few HFRS cases were reported. In Spain, seroprevalence of 0.06% was found in 2003 in more than 10,000 sera from blood donors using methods comparable to those used in our study [22], while other studies published in 2002 and 2009, based on smaller number of samples and using different methods for screening and confirmation, reported a seroprevalence of 0.31% and 2% respectively for distinct areas in Spain [23,24]. In Italy, a serological study on sera collected in 2002 in the north of the country from 488 forestry workers revealed no serum reaction to PUUV and, although a low seroprevalence for hantaviruses (0.4%) was found in bank voles [25]; no human cases of hantavirus infection were reported in Italy between 2000 and 2010 [4,26]. In Spain, only a few HFRS cases were reported during the same time period [4,26]. In Switzerland, seven cases of hantavirus infections were documented between 2003 and 2010 [14].

In 2012, eight new cases of hantavirus infections were reported to the Swiss Federal Office of Public Health (incidence: 0.1 per 100,000 population) [14]. Seven of the cases were confirmed, of whom five had been infected in an endemic region outside Switzerland (Nicole Gysin, Federal Office of Public Health, personnal communication, 10 December 2013). This is in contrast to the situation in Baden Württemberg, southwest Germany, where high numbers of infections were reported for 2012 (in weeks 1–17, n=501; incidence: 4.66 per 100,000 population) [27]. In Germany as a whole, seroprevalence of 1–2% was estimated 1995, while values of over 5% were documented more recently for highly endemic regions of Baden-Württemberg (2001) and Lower Bavaria (2009) [17-19]. Considerable differences between neighbouring regions have often been observed with hantavirus infections [18,19].

Our study highlights the difficulties arising from limited test specificity when investigating the hantavirus seroprevalence retrospectively in a population with a low incidence of infections. To ensure maximum sensitivity, we used two different ELISA kits for the screening. The hantavirus ELISA is based on a pool of recombinant nucleoprotein antigens and should detect antibodies against the most frequently detected European HFRS-causing hantaviruses. In our study, it is likely that this ELISA led to an excessively high rate of false-positive results, since very few of the 599 sera with positive results from the hantavirus ELISA could be confirmed by IFA or IBA. The reasons for this high proportion of non-specific reactions are unclear, but might be related to the recombinant antigens used or to problems associated with washing parameters of the automated ELISA system used for the study, although the assay quality controls were within the kit specifications. In other serological studies, higher serum dilutions were used for screening [24] or an increased cut-off value was proposed [28] to get around the problem of non-specific binding. The PUUV ELISA results for the same samples found that only 1% of the sera gave a positive OD ratio, indicating that this ELISA was less likely to generate false-positive results.

Of the 655 sera analysed by both ELISAs, only 30 could be confirmed by either IFA and/or IBA. Since none of the tested individuals reported symptoms that could be unequivocally attributed to a previous or current hantavirus infection, it seems impossible to determine whether they had been infected with hantaviruses or not. The FRNT, used as third method to further evaluate samples showing specific reaction to PUUV by IFA and IBA, is widely accepted as the gold standard for hantavirus serology of non-acute samples [21]. When an 80% reduction in FFU was applied as the cut-off, only two samples were positive at a serum dilution of 1:40 and two more samples achieved a 50% reduction in FFU. These four sera showed a strong signal in the IFA and IBA. Interestingly, no clear reduction in FFU was observed for any of the other sera analysed, which raises the question of whether the excellent specificity of the FRNT may be acquired at the expense of its sensitivity. For clinical infections, the presence of antibodies has been demonstrated up to 10–20 years after infection, using the FRNT [16,29]. Whether this would also be the case for subclinical infections is unknown. Furthermore, the selection of the virus strain used in the FRNT may influence the outcome. In our study, neutralisation was performed using a Russian PUUV strain (Kazan): the isolates circulating in central Europe may differ from this Russian isolate [30]. This may partially explain the fact that only sera with presumably high antibody titres led to a reduction in FFU. Interestingly, the neutralisation capacity of both sera that were positive in the neutralisation test was very similar for the two hantaviruses tested (PUUV and HTNV). This suggests that the PUUV strain used either differed substantially from the virus causing the infection and/or that some of the tested individuals might have been infected with another hantavirus species. The presence of Tula virus, for example, has been documented in Switzerland, in a 10 year-old boy bitten by a small wild rodent in 2000 as well as in rodents trapped between 2001 and 2009 [31,32].

Several laboratories in Europe use the IBA as diagnostic or confirmatory test [33,34]. This was also the method of choice in the earlier serological study performed in the north-eastern part of Switzerland [20]. This analysis of blood samples from 2002–03 revealed a seroprevalence of 0.5% among blood donors and in occupational risk groups, with a higher seroprevalence observed only in the small group of military personnel (2/103; 1.9%). When the same criterion (positive IBA) was used for confirmation, we found a slightly lower seroprevalence of 0.3% in the blood samples collected mainly in the central part of Switzerland. The rather minor difference in prevalence obtained in the two studies may result from our larger sample size, methodological differences when performing and interpreting the assay or it may reflect a truly higher prevalence in the population in the north-east of the country.

However, even a population-representative sample would scarcely allow to detect (small) foci and the high sample size needed would have been impracticable. Hence the reason we sampled a population residing in mainly rural areas, as living close to forested areas was found to be a risk factor [35].

Some recreational/leisure or work-related activities are known to be risk factors for PUUV infection [36-38]; but this was not found in our study. Travel to other endemic countries, such as in Asia or south-east Europe, was also not found to increase risk. Both findings might be explained by the low case numbers. An association of age (above 50 years) with seropositivity was found, which is consistent with observations from Sweden [39] based on seroepidemiology, but not with German findings, which were based on notification data including clinical symptoms [11]. This fact might be explained by the persistence and accumulation of hantavirus IgG antibodies in elderly persons in endemic regions, compared with those newly acquired in the course of a recent symptomatic infection within one epidemic season.

Although the large sample of blood donors was representative for the blood donor population in Switzerland, this study is limited by the fact that blood donors differ from the general population in several aspects, including health-related anthropometric and personality-related variables [40]. In addition, although the blood donors samples covered well the region of central Switzerland, we do not have data on donors in other regions of the country. Although blood donors may not represent an ideal control group for diseases related to environmentally, behaviourally or socially patterned exposures [39], due to the ease of accessibility of blood samples and personal information, blood donors are popular study populations for serological analysis. Serological data from blood donors may be used as basis value for comparative studies with population groups representing potential risk groups [20,41].

In our study, we compared the seroprevalence of blood donors residing in central Switzerland with that of young soldiers residing all over Switzerland. On the basis of prevalence data from a study performed in the north-eastern part of Switzerland in 2002–03, we investigated predominantly young soldiers as a potential risk group, but since their military service did not take place during a year of increased hantavirus activity in regions close to Switzerland, they were not at particular risk of infection [4,27,38]. Due to time-consuming preparatory work, the period of blood collection in the military personnel could not be handled flexibly and could not be postponed to the following year: in that year, increased numbers of hantavirus infections were documented in Germany [4,11]. Prevalence data from various populations, including blood donors, risk groups and symptomatic or asymptomatic volunteers from the general population, are available from several European countries, rendering them attractive for comparative analysis between different regions [18,22-24]. But, as we have shown, the methods applied to determine prevalence data need to be considered as well, when comparing different studies, since the influence of the test sensitivity and specificity on the determined seroprevalence may be substantial.

In summary, we have found a low prevalence of hantavirus infections in the study populations, but the periodic hantavirus epidemics in neighbouring countries requires attention of public health authorities and measures of preparedness including active surveillance need to be evaluated. Furthermore, at-risk populations – known from other studies to be people living in rural areas and people carrying out activities in areas and facilities infested by rodents – should be informed about potential exposure risk to hantaviruses and should be advised regarding precaution measures and symptoms.

Acknowledgements

This study was supported by the Swiss Federal Office for Civil Protection. We are indebted to all field study and laboratory personnel for their enthusiasm at work and our thanks include blood donors and military people who participated and all those who supported this study. We would like to acknowledge the companies Euroimmun, Progen, Focus and Mikrogen for their helpful comments.

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