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SHORT COMMUNICATION

Evaluation of the public health risk for autochthonous transmission of mosquito-borne viruses in southern Switzerland

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Abstract. Epidemics of mosquito-borne diseases such as chikungunya and dengue fever are becoming more frequent around the world. In Switzerland, autochthonous cases have not been reported so far, although the presence of the vector *Aedes albopictus* in urban areas of southern Switzerland increases the risk of indigenous transmissions subsequent to imported cases. In 2018, the potential risk of an outbreak of arboviral diseases was assessed in five municipalities of southern Switzerland. The population abundance of *Ae. albopictus* was evaluated during the mosquito active season by the mean number of *Ae. albopictus* bites per day per person (estimated using the human landing collection method) and the risk of outbreak in the case of the introduction of chikungunya, dengue or Zika viruses was estimated. In the five localities investigated, no epidemic risk appeared to be present for any of the arboviruses taken into consideration in the initial months (i.e. mid-May to end of July) of *Ae. albopictus* activity. In the case of the introduction of chikungunya (mutated or not), dengue (serotype 1) or Zika (African lineage) viruses during mid-end August, an epidemic could have occurred in all the municipalities investigated. In mid-end September, the introduction of same arboviruses could have led to an epidemic in three of the five municipalities investigated.

Key words. *Aedes albopictus*, basic reproduction number R_0, human landing collection, mosquito-borne viruses.

Epidemics of mosquito-borne diseases, such as chikungunya (CHIKV), dengue (DENV) and Zika (ZIKV) viruses, are becoming more frequent around the world (Marklewitz & Junglen, 2019). The 2015 outbreak of ZIKV is the latest demonstration of how arboviruses are re-emerging globally (Kindhauser *et al.*, 2016). Factors such as global trade and climate change favour the spread of the highly adaptable and invasive mosquito vectors, with infected travellers carrying the fast evolving viruses around the world within no time (Quam *et al.*, 2015; Liu-Helmersson *et al.*, 2016; Massad *et al.*, 2018; Lillepold *et al.*, 2019). The threat of arboviral infections has recently reached continental Europe, with several locally transmitted CHIKV and DENV cases occurring over the past decade (Barzon, 2018). In 2017, epidemics of CHIKV occurred in France and in Italy involving strains of different origin and therefore they were not related (Lindh *et al.*, 2018). In early October 2018, three cases of autochthonous DENV were confirmed in Spain, as well as eight in France, in three separate outbreaks (European Centre for Disease Prevention and Control, 2018).

In Switzerland, autochthonous cases of CHIKV and DENV have not been reported so far. However, the number of imported cases increases regularly as in other European countries: in 2016, the number of reported DENV cases in Switzerland reached 201, whereas, for CHIKV, a peak of imported cases was detected in 2014 with 78 cases (https://www.bag.admin.ch). The presence of *Ae. albopictus* in densely populated urban areas of southern Switzerland (Flacio *et al.*, 2015, 2016) increases the risk of indigenous transmissions subsequent to imported cases during the summer season.

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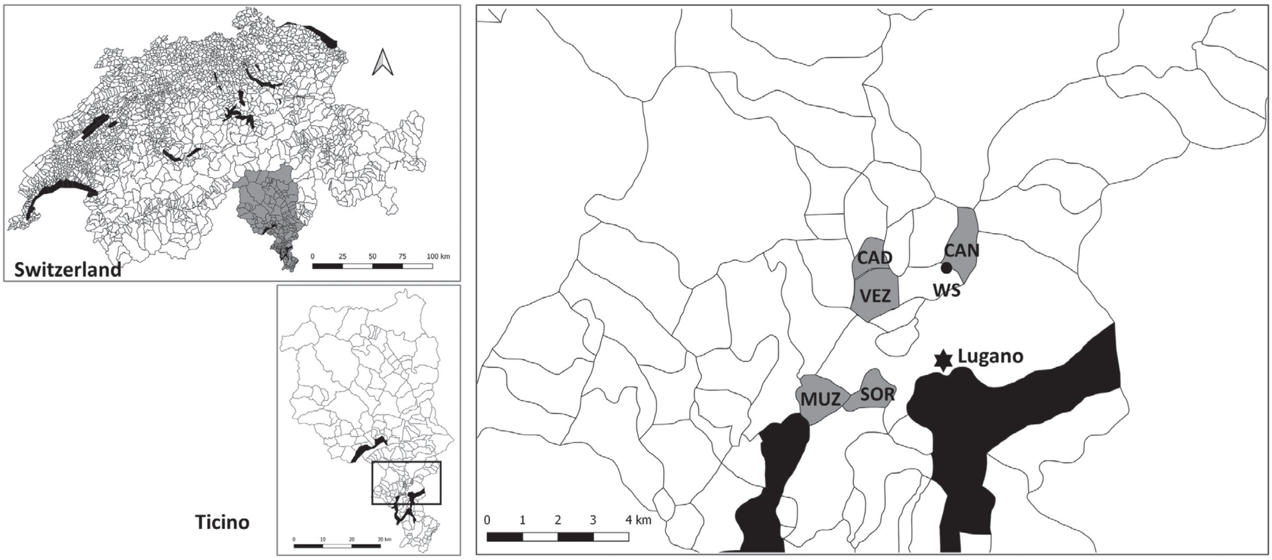

Fig. 1. Canton of Ticino with the five municipalities investigated: Cadempino (CAD), Canobbio (CAN), Muzzano (MUZ), Sorengo (SOR) and Vezia (VEZ). WS, weather station.

The risk of autochthonous transmission of CHIKV, DENV and ZIKV has been estimated in European countries where *Ae. albopictus* is established, such as Italy (Carrieri *et al.*, 2012; Guzzetta *et al.*, 2016a, 2016b; Manica *et al.*, 2017), Greece (Bellini *et al.*, 2016) and Montenegro (http://project-lovcen.me). Several factors affect the likelihood of an arboviral outbreak, such as the density of human and mosquito populations, the introduction of diseases by infected travellers, the seasonal temperature (which in turn affects the mosquito gonotrophic cycle and survival rate, as well as the extrinsic incubation period of the virus in the vector) and the competence of the local mosquito population to transmit the virus, etc. For example, in Italy, the risk of autochthonous transmission by *Ae. albopictus* is predicted to be low to significant depending on the virus, the season and the location (Carrieri, 2012; Guzzetta *et al.*, 2016a, 2016b; Manica *et al.*, 2017). The present study assessed the risk of an outbreak of CHIKV, DENV and ZIKV in southern Switzerland where the vector *Ae. albopictus* is established, aiming to help decision-making for the timely adoption of intervention measures in the occurrence of imported cases.

The risk of autochthonous transmission was assessed monthly during the 2018 *Ae. albopictus* reproduction season (mid-May to end of September) in five municipalities (i.e. Cadempino, Canobbio, Muzzano, Sorengo and Vezia) located in the surroundings of Lugano (N46.003428, E8.951320), Switzerland (Fig. 1). The localities were selected according to the presence and abundance of *Ae. albopictus* from the 2017 surveillance data and had a similar urban landscape and housing type. The population density of *Ae. albopictus*, one of the key factors affecting the risk of autochthonous transmission of arboviruses, was assessed in the different localities by two indices: the mean number of female mosquito bites per day per person [estimated through the human landing collection (HLC) method] and the mean number of eggs laid by female mosquitoes per day (estimated by egg collections using ovitraps). HLCs were performed during 17–25 May (HLC1), 21–29 June (HLC2), 18–26 July (HLC3), 20–24 August (HLC4) and 17–22 September (HLC5). Exposure for 15 min in the most favourable conditions with respect to being bitten (i.e. standing without repellent or protecting dresses, with only arms and legs exposed, in the most suitable shaded position to attract host-seeking *Ae. albopictus* females) can be assumed as an estimate of the number of bites a person can receive per day (Carrieri *et al.*, 2012). Five HLC sampling stations were established in each municipality. Collections were performed by five trained biologists by means of manual aspirators during the *Ae. albopictus* peak activity (16.00 to 18.00 h). During this 2-h period, each operator conducted five HLC sessions of 15 min each, rotating between stations of a municipality. After each 15-min session, collected adult female mosquitoes were identified, counted and released back to the field (Carrieri *et al.*, 2012). During the next day of collection, each operator repeated the collection in a different locality, until all operators had covered all the municipalities. HLCs were performed to achieve synchronous sessions in the five municipalities and by rotating operators each day.

Egg collections were carried out using ovitraps (Flacio *et al.*, 2015) positioned in vegetated shaded sites near each HLC station. The wooden paddles in the ovitraps were recovered and replaced with new ones every 14 days by municipal workers, resulting in nine consecutive checking rounds. For comparison between number of eggs laid in ovitraps and number of bites estimated by HLC, the ovitrap data obtained from checking rounds carried out in the 2 weeks preceding the HLC periods were considered (Carrieri *et al.*, 2012). Paddles recovered from ovitraps were brought to the laboratory and the number of eggs on paddles was counted. The number of eggs laid in each ovitrap was divided by the number of days the ovitrap was active in the field to obtain the number of eggs per day. Significant differences between number of female mosquitoes captured by HLC operators, number of female mosquitoes captured by

Table 1. Values calculated or obtained from the literature for the parameters involved in the calculation of R_0.

Parameter	Value	Reference
h: proportion of human blood meals (host feeding pattern)	0.86	Valerio *et al.* (2010)
GC: duration (number of days) of the gonotrophic cycle	HLC1: 11.92 d	Calculated in function of temperatures∗according to the model of Poletti *et al.* (2011)
	HLC2: 6.48 d	
	HLC3: 5.8 d	
	HLC4: 5.21 d	
	HLC5: 7.76 d	
S_m: vector competence of *Ae. albopictus*	Non-mutated CHIKV and CHIKV A226V mutant: 0.80	Fortuna *et al.* (2018)†
	DENV1: 0.67	Vega-Rua *et al.* (2013)
	DENV2: 0.72	Mitchell (1991)
	DENV3: 0.64	Mitchell (1991)
	DENV4: 0.34	Mitchell (1991)
	ZIKV Uganda: 1.00	Wong *et al.* (2013)
	ZIKV Asian: 0.29	Di Luca *et al.* (2016)
1/*V*: viraemia	CHIKV: 0.17	Peters & Dalrymple (1990)
	DENV2: 0.25 ZIKV: 0.2	Böelle *et al.* (2008) Gubler *et al.* (1981); Wong *et al.* (2013)
S_v: population susceptibility	1	
p: female daily survival rate	0.95	http://project-lovcen.me/
i: extrinsic incubation period	CHIKV: 0.71 GC	Carrieri *et al.* (2012)
	DENV2: 2 GC	Barbazan *et al.* (2010)
	ZIKV: 1.5 GC	Wong *et al.* (2013)

∗Mean temperatures in the study period were 16.66 °C (HLC1), 22.2 °C (HLC2), 23.19 °C (HLC3). 24.2 °C (HLC4) and 20.6 °C (HLC5).

†According to competence analyses by Fortuna *et al.* (2018), the transmission rates of the chikungunya strain with the *Ae. albopictus*-adaptive E1:A226V mutation (2007 outbreak in Emilia Romagna) and without this mutation (2017 outbreak in Lazio and Calabria regions) do not show any significant difference.

HLC, human landing collection; CHIKV, chikungunya; DENV, dengue; ZIKV, Zika.

HLC in the different locations and number of eggs in the different locations were tested using nonparametric Kruskal Wallis *H*-tests with post-hoc pairwise comparisons between groups, with Bonferroni correction. Data were $\log_{10}(x+1)$ transformed. The mean number of biting *Ae. albopictus* females collected by HLC operators in all the localities was compared with the mean egg density detected in the 2 weeks preceding each HLC by Spearman correlation. The same correlation was calculated with egg density corrected according to the population density known for each area because the number of bites received by one person depends on the mean number of females per hectare, as well as on the human population density per hectare. The data were corrected by dividing the mean egg density by the number of people per hectare, which was considered stable during the whole mosquito season in the five municipalities studied. Statistical analyses were carried out using SPSS, version 24 (IBM Corp., Armonk, NY, U.S.A.).

To evaluate the risk of autochthonous transmission, the basic reproduction number R_0 (defined as the number of secondary disease cases that originate from a primary case) was calculated using McDonald's equation as modified by Fine (1981) and Reisen (1989):

$$R_0 = \frac{ma^*(a\, S_m V\, S_v p^i)}{(-\log_e p)}$$

where *ma* is the mean number of bites per day per person. When $R_0 < 1$, only accidental and isolated cases can occur, whereas, for an epidemic to occur, R_0 has to be >1. All of the values for the parameters that were used in the calculation of the epidemic risk threshold are indicated in Table 1 and the detailed description of the parameters is provided in Carrieri *et al.* (2012). Briefly, the parameter a corresponds to *h*/*GC*, where *h* is the proportion of human blood meals (host feeding pattern) and *GC* is the duration in number of days of the gonotrophic cycle. S_m is the competence of *Ae. albopictus* for a determined virus. *V* is the period during which the viraemia in the infected host is sufficient to infect the female mosquito. Because the antibody level in the study site inhabitants is likely to be near to 0%, a value of 1 was considered for the proportion of the human population sensitive to the infection (S_v). The value of 0.95 for female daily survival rate (*p*) in wet period (< 10 days without precipitations) in northern Italy (http://project-lovcen.me) was taken has reference value for the present study; *i* is the duration of the extrinsic incubation period of the virus in the vector. Basic reproduction numbers R_0 were calculated using EXCEL 2016 (Microsoft Corp., Redmond, Washington). Meteorological data (i.e. hourly records of temperature at 2 m from ground, mean daily temperature and precipitation) were obtained from a permanent weather station in Canobbio (data source: MeteoSwiss; https://www.meteoswiss.admin.ch/home.html), which was located within 5 km of the municipalities investigated (Fig. 1).

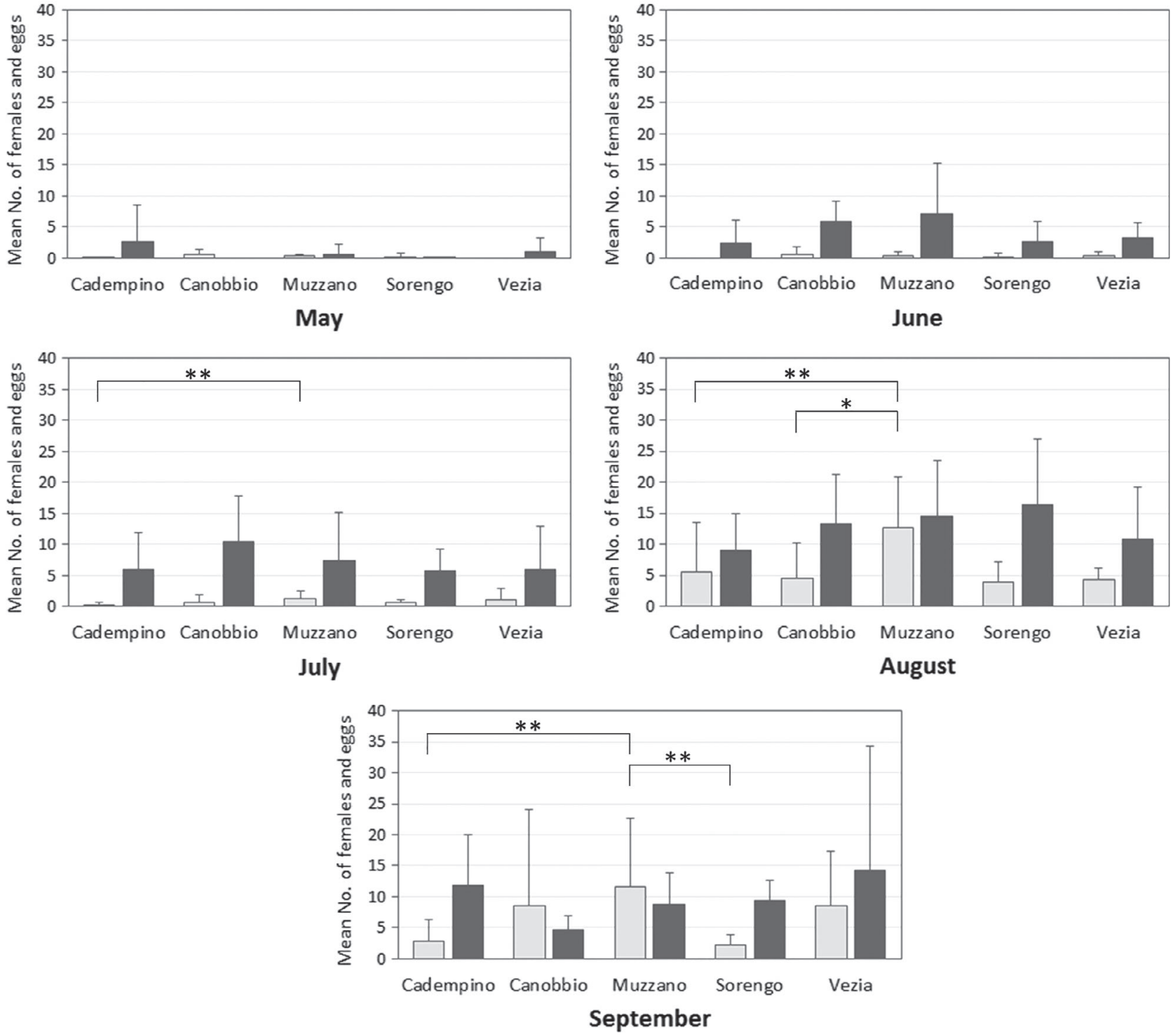

Fig. 2. Mean number of *Aedes albopictus* biting females per person per day (light grey bars) collected in each human landing collection period and municipality and mean number of mosquito eggs per ovitrap per day (dark grey bars) collected in the corresponding ovitrap checking round. $*P < 0.05$, $**P < 0.001$. Standard deviations are represented by black bars.

The number of female mosquito captured followed the same temporal trend in the municipalities examined (Fig. 2). Few females were captured in May (0.22 females on average between municipalities during HLC1), June (0.33 females during HLC2) and July (0.80 females during HLC3). As expected, more females were collected at the end of August (6.18 females during HLC4) and in mid-September (6.76 females in HLC5), corresponding to the seasonal peak of the *Ae. albopictus* population density (Flacio *et al.*, 2016). The number of female mosquitoes captured for each separate HLC period and locality did not significantly differ between the five HLC operators, except in one case where a significant difference between operators was found in Muzzano during HLC3 ($\chi^2 = 11.552$, d.f. = 4, $P < 0.05$). Pairwise comparison highlighted a significant difference ($P < 0.05$) between two of the operators. In the specific case, one operator collected a mean of three females, whereas the other operator did not capture any females. The mean number of biting mosquitoes, for separate HLC periods and pooled operator results, did not differ between the five locations for HLC1 ($\chi^2 = 7.347$, d.f. = 4, $P = 0.119$) and HLC2 ($\chi^2 = 4.332$, d.f. = 4, $P = 0.363$). A significant difference was found during HLC3 ($\chi^2 = 13.249$, d.f. = 4, $P < 0.01$), HLC4 ($\chi^2 = 14.689$, d.f. = 4, $P < 0.01$) and HLC5 ($\chi^2 = 20.737$, d.f. = 4, $P < 0.001$). During HLC3, HLC4 and HLC5, the mean number of *Ae. albopictus* females collected in Muzzano was significantly higher ($P < 0.01$) than in Cadempino (Fig. 2). For HLC4, the mean number of *Ae. albopictus* females collected in Muzzano was also significantly

Table 2. Rates of increase of disease (R_0) estimated for different viruses in the five localities included in the present study.

	Non-mutated CHIKV and CHIKV A226V mutant			DENV1			ZIKV Uganda			ZIKV Asian		
	Lower 95% CI	R_0	Upper 95% CI	Lower 95% CI	R_0	Upper 95% CI	Lower 95% CI	R_0	Upper 95% CI	Lower 95% CI	R_0	Upper 95% CI
HLC1 (17–25 May)												
Cadempino	0.00	0.00	0.01	0.00	0.00	0.01	0.00	0.00	0.01	0.00	0.00	0.00
Canobbio	0.00	0.05	0.10	0.00	0.03	0.06	0.00	0.04	0.09	0.00	0.01	0.03
Muzzano	0.00	0.04	0.09	0.00	0.02	0.05	0.00	0.04	0.08	0.00	0.01	0.02
Sorengo	0.00	0.02	0.07	0.00	0.01	0.04	0.00	0.02	0.06	0.00	0.01	0.02
Vezia	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
HLC2 (21–29 June)												
Cadempino	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Canobbio	0.00	0.17	0.35	0.00	0.13	0.28	0.00	0.19	0.39	0.00	0.05	0.11
Muzzano	0.00	0.10	0.22	0.00	0.08	0.18	0.00	0.11	0.25	0.00	0.03	0.07
Sorengo	0.00	0.07	0.16	0.00	0.05	0.13	0.00	0.08	0.18	0.00	0.02	0.05
Vezia	0.00	0.10	0.26	0.00	0.08	0.21	0.00	0.11	0.30	0.00	0.03	0.09
HLC3 (18–26 July)												
Cadempino	0.00	0.05	0.11	0.00	0.04	0.10	0.00	0.06	0.13	0.00	0.02	0.04
Canobbio	0.02	0.23	0.43	0.02	0.19	0.36	0.03	0.27	0.51	0.01	0.08	0.15
Muzzano	0.15	0.41	0.67	0.12	0.34	0.56	0.17	0.47	0.78	0.05	0.14	0.23
Sorengo	0.03	0.20	0.38	0.03	0.17	0.32	0.04	0.24	0.44	0.01	0.07	0.13
Vezia	0.07	0.39	0.72	0.05	0.33	0.61	0.08	0.46	0.84	0.02	0.13	0.24
HLC4 (20–24 August)												
Cadempino	0.33	1.99	3.64	0.29	1.73	3.18	0.39	2.37	4.34	0.11	0.69	1.26
Canobbio	0.50	1.64	2.78	0.43	1.43	2.43	0.59	1.95	3.31	0.17	0.57	0.96
Muzzano	2.22	3.94	5.67	1.94	3.44	4.94	2.64	4.70	6.75	0.77	1.36	1.96
Sorengo	0.44	1.41	2.37	0.39	1.23	2.07	0.53	1.67	2.82	0.15	0.49	0.82
Vezia	0.75	1.57	2.38	0.65	1.37	2.08	0.89	1.86	2.84	0.26	0.54	0.82
HLC5 (17–22 Sep)												
Cadempino	0.20	0.65	1.09	0.15	0.48	0.81	0.22	0.69	1.17	0.06	0.20	0.34
Canobbio	0.01	1.91	3.81	0.01	1.41	2.81	0.02	2.06	4.10	0.00	0.60	1.19
Muzzano	1.05	2.63	4.20	0.78	1.94	3.10	1.13	2.82	4.51	0.33	0.82	1.31
Sorengo	0.20	0.48	0.76	0.15	0.35	0.56	0.21	0.51	0.81	0.06	0.15	0.24
Vezia	0.74	1.89	3.03	0.55	1.39	2.23	0.80	2.03	3.25	0.23	0.59	0.94

No colour: $R_0 < 1$ (no risk); light grey: $1 \leq R_0 < 2$ (low risk); medium grey: $2 \leq R_0 < 3$ (moderate risk); dark grey: $R_0 \geq 3$ (high risk). Confidence interval (CI) at a confidence level of 95%: estimate of the range of values, with the 95% probability that a parameter will fall within the range estimate. HLC, human landing collection; CHIKV, chikungunya; DENV, dengue; ZIKV, Zika.

higher than in Canobbio ($P < 0.05$), whereas, during HLC5, significantly more females were collected in Muzzano than in Sorengo ($P < 0.01$).

In general, more eggs were recovered at the beginning of August and September (Fig. 2). This corresponds to an activity peak in 2018 *Ae. albopictus* density going from mid-August to mid-September. Therefore, the peak period of oviposition on ovitraps in the 2 weeks preceding the different HLC periods corresponds with the peak of adult *Ae. albopictus* females collected by HLC. The mean number of eggs laid by *Ae. albopictus* female mosquitoes in each checking round did not statistically differ between locations (May: $\chi^2 = 2.157$, d.f. = 4, $P = 0.707$; June $\chi^2 = 3.713$, d.f. = 4, $P = 0.446$; July: $\chi^2 = 2.095$, d.f. = 4, $P = 0.718$; August: $\chi^2 = 2.732$, d.f. = 4, $P = 0.604$; September: $\chi^2 = 5.582$, d.f. = 4, $P = 0.233$).

To estimate the risk of epidemics, an essential parameter is the mean number of *Ae. albopictus* bites per human per day, which can be estimated by HLC. In northern Italy, a good correlation was observed between the number of host-seeking *Ae. albopictus* females derived from HLC and the number of eggs in ovitraps, allowing this last index to be used for the calculation of the rate of increase of a disease (basic reproduction number) R_0 (Carrieri, 2012; Carrieri *et al.*, 2012). The use of ovitraps for the estimation of the adult mosquito abundance is the easiest and least expensive method, and would therefore be the most suitable for determination of disease risk thresholds. In addition, this method is already used for monitoring the tiger mosquito in southern Switzerland. In the present study, a Spearman's rank order correlation was run to determine the relationship between the mean number of *Ae. albopictus* biting females per human per day and the corresponding mean number of eggs per day. A significant moderate positive correlation was found between the two variables [$r_s = 0.747$, $n = 25$, $P < 0.01$]. However, the coefficient of determination r^2 was relatively low (36.5%), indicating that knowledge of one of the variables would account for only 36.5% of the variation in the other. A slightly higher r^2 (46.6%) was obtained after the number of eggs per ovitrap per day was corrected according to the human population density known for each area. Consequently, despite the correlation between the variables being significant, it could not be considered valuable for predictive purposes, indicating that data from ovitraps could not be used for the estimation of the adult mosquito abundance and thus for the determination of disease risk thresholds. The risk of outbreak in the case of the introduction of CHIKV, DENV and ZIKV throughout viremic travellers was therefore estimated based on HLC data.

Basic reproduction numbers R_0 calculated for the five municipalities included in the present study are reported in Table 2. In mid-end May (HLC1), June (HLC2) and July (HLC3), the risk for epidemics for all viruses analysed was absent, with possibly only isolated cases. In the case of the introduction of CHIKV (mutated or not), DENV (serotype 1) or ZIKV (African lineage) during mid-end August (HLC4), an epidemic could have occurred in all the municipalities investigated. In mid-end September (HLC5), the introduction of the same arboviruses could have led to an epidemic in three (i.e. Canobbio, Muzzano and Vezia) of the five municipalities investigated. These results are similar to the results of risk assessment conducted in 2007 and 2008 in the Emilia-Romagna region of Italy (Carrieri *et al.*, 2012), where $> 20\%$ of the urban areas had an outbreak risk for the mutated CHIKV similar to that calculated for the 2007 epidemic ($R_0 > 3$) and Montenegro (http://project-lovcen.me).

The choice of different vector competence values for specific arboviruses can influence the outcomes of the R_0 formula (although the same reasoning also applies to other parameters) and, consequently, the estimation of risk of outbreak. The present risk assessment used vector competence values obtained by Fortuna *et al.* (2018), where transmission rates of the chikungunya strain with the *Ae. albopictus*-adaptive E1:A226V mutation (2007 outbreak in Emilia Romagna) and without this mutation (2017 outbreaks in Lazio and Calabria regions) did not differ significantly, in contrast to that obtained in other studies (Mitchell, 1991). The vector competence of *Ae. albopictus* for specific virus strains in southern Switzerland mosquito populations has yet to be determined and experiments are currently in progress to fill this gap.

Aedes albopictus is a sedentary species, flying actively over short distances from its breeding site (about 50–200 m) (Marini *et al.*, 2010). Therefore, the high abundance of adults is related to the proximity of important breeding hotspots. Indeed, during HLC, evident *Ae. albopictus* breeding hotspots were observed near the sampling sites in Canobbio, Muzzano and Vezia. These hotspots were not removed for scientific correctness. Highly productive breeding sites are usually found on private lands and their presence is not homogenous within the territory, depending a great deal on the effectiveness of interventions by citizens. The results obtained in the present study indicate that the risk for epidemic exists mainly in the presence of important breeding hotspots. In southern Switzerland, mosquito control methods (e.g. larvicide treatments of stormwater catch basins) in public areas of municipalities colonised by *Ae. albopictus* are well established (Flacio *et al.*, 2015). Education and community participation for breeding source reduction on private lands are also crucial and awareness campaigns are promoted every year aiming to encourage citizen participation in mosquito control, although the effectiveness of the community-based approach still needs to be evaluated. Only the continued surveillance and control of the tiger mosquito and the pathogens that it can transmit, as well as an evaluation of the epidemic risk in real-time, will prevent or reduce the risk of the spread of arboviruses in Switzerland.

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