Emergence of sandflies (Phlebotominae) in Austria, a Central European country

Wolfgang Poeppl · Adelheid G. Obwaller · Martin Weiler · Heinz Burgmann · Gerhard Mooseder · Susanne Lorentz · Friedrich Rauchenwald · Horst Aspöck · Julia Walochnik · Torsten J. Naucke

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Abstract The possible existence of autochthonous sandfly populations in Central Europe north of the Alps has long been excluded. However, in the past years, sandflies have been documented in Germany, Belgium, and recently, also in Austria, close to the Slovenian border. Moreover, autochthonous human Leishmania and Phlebovirus infections have been reported in Central Europe, particularly in Germany. From 2010 to 2012, sandfly trapping (740 trap nights) was performed at 53 different capture sites in Austria using battery-operated CDC miniature light traps. Sites were chosen on the basis of their climate profile in the federal states Styria, Burgenland, and Lower Austria. Sandfly specimens found were transferred to 70 % ethanol for conservation. Identification was based on morphological characters of the male genitalia and the female spermathecae, respectively. Altogether, 24 specimens, 22 females and 2 males, all identified as Phlebotomus (Transphlebotomus) mascitti Grasi, 1908, were found at six different sampling sites in all three federal states investigated. The highest number of catches was made on a farm in Lower Austria. Altogether, the period of sandfly activity in Austria was shown to be much longer than presumed, the earliest capture was made on July 3rd and the latest on August 28th. Sandflies have been autochthonous in Austria in small foci probably for long, but in the course of global warming, further spreading may be expected. Although P. mascitti is only an assumed vector of Leishmania spp.—data on its experimental transmission capacity are still lacking—the wide distribution of sandflies in Austria, a country thought to be free of sandflies, further supports a potential emergence of sandflies in Central Europe. This is of medical relevance, not only with respect to the transmission of Leishmania spp. for which a reservoir is given in dogs, but also with respect to the phleboviruses.

Introduction

Sandflies (Diptera: Psychodidae: Phlebotominae) serve as vectors of various pathogens, including Leishmania spp., Bartonella spp. and phleboviruses. In Europe, sandflies are typical Mediterranean faunal elements which are widely distributed mainly in southern parts of Europe, but also in extra-Mediterranean regions in Eastern and Western Europe.
Austria is a landlocked, largely mountainous Central European country of 83,860 km² located within the alpine arch. The lowlands and plains of the east and southeast, however, are part of the Vienna basin and the Pannoni low country with continental climate characterised by hot summers and cold winters. Austria is bordered by the Czech Republic and Germany to the north, Hungary and Slovakia to the east, Slovenia and Italy to the south, and Switzerland and Liechtenstein to the west, and is divided into nine federal states.

In July 2010, July and August 2011 and 2012, entomological field studies were carried out in Southern and Eastern Austria to investigate the possible occurrence of sandflies. Battery-operated CDC miniature light traps using fine gossamer collection bags (model #512, John W. Hock Company, Gainesville, Florida) were run from dusk to dawn. Bags were collected daily in the morning and the contents were stored at −20 °C until inspection. Sandfly specimens found were transferred to 70 % ethanol for conservation. For species identification, sandflies were cleared in potassium hydroxide solution, and finally, slide-mounted in Hoyer’s fluid (Naucke 2000). Temperature data on trapping regions were retrospectively obtained from the Central Institute for Meteorology and Geodynamics (ZAMG).

Materials and methods

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nights (July 14th to 16th 2010) with two traps. The traps were placed in a shed with loamy ground which was used as a lumber storage. In the area where those three villages with positive capture results are located, the mean monthly temperature in July 2010 was 18.9 °C at 7 a.m., 26.9 °C at 2 p.m. and 23.4 °C at 7 p.m.

In Burgenland, one female was found in Deutschkreutz (47°35′45″ N/16°37′43.2″ E at 196 m.a.SL) during trapping for a single night in August (August 2nd 2011) using three traps. The traps had been set up near human dwellings where no animals were kept. In the area of Deutschkreutz the mean monthly temperature in August 2011 was 18.5 °C at 7 a.m., 25.9 °C at 2 p.m. and 21.8 °C at 7 p.m. Further sandflies, namely one female and one male were captured in Luising (47°0′40.0″ N/16°28′46.6″ E; 47°0′45.9″ N/16°28′50.2″ E at 233 m.a.SL), where four traps had been in use from July 18th to 20th and August 2nd to 6th. One female and one male were captured on the 3rd and 19th of August 2011, respectively. The trap sites were located near a human dwelling in a wooden shed with little organic matter on the floor, in a barn with concreted floor used for storage of equipment and straw bales and in an open garage with concrete pavement used for storage. A dog and several cats were held at this farm. In this area, the mean monthly temperature in August 2011 was 18.5 °C at 7 a.m., 26.3 °C at 2 p.m. and 22.2 °C at 7 p.m. In Luising, sandfly trapping was repeated 1 year later for 34 days between July 3rd and August 31st 2012 using six traps. Two females were captured between July 3rd and August 7th. A female and a male captured in Rohrau are shown in Figs. 2 and 3, respectively. The mean monthly temperature in this area was 19.5 °C at 7 a.m., 26.6 °C at 2 p.m. and 22.7 °C at 7 p.m. in July 2012 and 19.2 °C at 7 a.m., 26.9 °C at 2 p.m. and 23.6 °C at 7 p.m. in August 2012.

Discussion

Until the first and hitherto unique finding of sandflies in Austria near the Carinthian/Slovenian border in the year 2009 (Naucke et al. 2011), Austria was believed to be free of sandflies. However, it had already been shown that only a moderate increase in temperature would lead to conditions favourable for the occurrence of sandflies in large parts of the country (Aspöck et al. 2006). The expected species were P. mascittii in western parts of Austria and Phlebotomus neglectus in the east (Aspöck et al. 2006). A more recent study investigating the responses of sandfly vector species to climate change in Europe
| Locality name (latitude, longitude, altitude*) | Captures | Capture date | Capture period | Mean monthly evening temperature (7 p.m.) | Trap site description |
|-----------------------------------------------|----------|--------------|----------------|------------------------------------------|----------------------|
| **Burgenland**                                |          |              |                |                                          |                      |
| Deutschkreutz *(47°35′45″ N, 16°37′43.2″ E, 196″)* | 1 female | 2 August 2011 | 2 August 2011 | 21.8 °C | Near human dwelling, No livestock, No companion animals |
| Luising *(47°0′40.0″ N, 16°28′46.6″ E, 233) | 1 male, 1 female | 3 August 2011, 19 August 2011 | 18–20 July 2011, 1–26 August 2011 | 21.3 °C, 21.7 °C | Near human dwelling with chickens; (a) Wooden shed with organic matter on the floor, (b) Barn with concrete floor, (c) Open garage with concrete pavement used for storage |
| *(47°0′45.9″ N, 16°28′50.2″ E, 233) | 2 females | 24 July 2012, 20 August 2012 | 2 July–31 August 2012 | 23.0 °C/23.2 °C |                      |
| **Lower Austria**                             |          |              |                |                                          |                      |
| Rohrau *(48°3′56.664″ N, 16°51′33.084″ E, 148)* | 3 females | 24 August 2011, 28 August 2011 | 16–19 August 2011, 22–28 August 2011 | 22.2 °C, 22.7 °C/23.6 °C | Near human dwelling; dog and cats; (a) Wooden barn, (b) Open, concreted garage used for storage |
| *(33.084″ E, 148)* | 1 male, 6 females | 2 July 2012, 6 July 2012, 11 July 2012, 25 July 2012, 7 August 2012 |                      |                      |                      |
| **Styria**                                    |          |              |                |                                          |                      |
| Halbenrain *(46°43′21.18″ N, 15°56′30.96″ E, 216)* | 5 females | 14 July 2010, 15 July 2010 | 12–16 July 2010 | 26.9 °C | Wooden shed near human dwelling |
| *(46°43′35.04″ N, 15°49′4.26″ E, 233)* | 1 female | 14 July 2010 | 12–16 July 2010 | 26.9 °C | Open garage, concrete pavement near human dwelling |
| Pfärrsdorf *(46°4′22′.84″ N, 15°57′51.12″ E, 216)* | 3 females | 14 July 2010, 15 July 2010 | 12–16 July 2010 | 26.9 °C | Shed with soil bottom near human dwelling |
| Ratzenau *(46°4′35.04″ N, 15°49′4.26″ E, 233)* |                      |              |                |                                          |                      |

*Altitude in metres above sea level
by combining their dispersal abilities with climatic prospects, projected sandfly establishment of species with current south-eastern focus (P. neglectus, P. perflieiwi) in eastern Austrian regions neighbouring Slovenia and Hungary in the near future (Fischer et al. 2011). In that study it was further projected that P. ariasi and P. mascittii would disperse to south-eastern parts of Austria and would be able to occur in eastern and north-eastern parts at the end of the twenty-first century (Fischer et al. 2011). Thus, it appears surprising that in the present study (1) eastern focus (Fig. 3) and not (2) P. mascittii was found among 883 sandflies (Berdjane-Brouk et al. 2011; Maroli and Bettini 1977; Veronesi et al. 2007). Since the first description of P. mascittii in 1908 (Grassi 1908), not more than 20 P. mascittii were trapped each season (Raynal 1954). There is only one place known in Europe, where P. mascittii can be found in high numbers, and this is a 450-m long and blocked railway tunnel on the island of Corsica (Toumanoff and Chassignet 1954). Adult P. mascittii can be found all through the year inside this tunnel (Naucke et al. 2008b). In Germany, a 200-year-old barn could be identified as a breeding place (Naucke 2002). Thus, to evaluate realistic population densities of P. mascittii, optimised catching methods are required. In a recent study from Northern Italy comparing sticky and sucking traps with or without light bait over a 4-month sampling period, only one out of 2,253 sandflies was identified as P. mascittii (Veronesi et al. 2007). Likewise, in an entomological study performed with CDC miniature light traps in Northern Algeria, only one individuals of P. mascittii was found among 883 sandflies (Berdjane-Brouk et al. 2011). However, because a sandfly species is probably not survivable in such a low-population density, the number of catches made in the present study apparently does not reflect the real population density of P. mascittii in Austria. Our personal observation based on sandfly breeding experiences is that P. mascittii is less attracted by light in contrast to other sandfly species. Escaped

P. mascittii is widely distributed in the Mediterranean region from Spain in the West to Turkey in the East. North of the Alps, it has been found in France, Switzerland, Belgium, Germany (Aspöck et al. 2008), and recently in the South of Austria (Naucke et al. 2011). Considering the wide distribution, the presence of such remnants of the Holocene optimum about 6,500 years ago in very localised spots in Austria is not surprising (Aspöck 2008; Aspöck 2010).

Based on the results of the present study, it can be concluded that sandflies are more widely distributed (and most probably have been for long) in Central Europe than previously realised. Sandflies were captured at 6 out of 44 trapping sites and in most areas trapping was performed only for a short period of time (in some areas even for only a single night). Thus, the present study provides clear evidence for the permanent occurrence of sandflies in those areas, but care should be taken when considering areas with negative capture results as sandfly-free. Nonetheless, the total number of sandfly catches made was rather low. Even at catching sites were sandfly trapping was performed over a period of several weeks, only a handful catches were made. In line with this, in several entomological studies, P. mascittii was found at low densities (Berdjane-Brouk et al. 2011; Maroli and Bettini 1977; Veronesi et al. 2007). Since the first description of P. mascittii in 1908 (Grassi 1908), not more than 20 P. mascittii were trapped each season (Raynal 1954). There is only one place known in Europe, where P. mascittii can be found in high numbers, and this is a 450-m long and blocked railway tunnel on the island of Corsica (Toumanoff and Chassignet 1954). Adult P. mascittii can be found all through the year inside this tunnel (Naucke et al. 2008b). In Germany, a 200-year-old barn could be identified as a breeding place (Naucke 2002). Thus, to evaluate realistic population densities of P. mascittii, optimised catching methods are required. In a recent study from Northern Italy comparing sticky and sucking traps with or without light bait over a 4-month sampling period, only one out of 2,253 sandflies was identified as P. mascittii (Veronesi et al. 2007). Likewise, in an entomological study performed with CDC miniature light traps in Northern Algeria, only one individuals of P. mascittii was found among 883 sandflies (Berdjane-Brouk et al. 2011). However, because a sandfly species is probably not survivable in such a low-population density, the number of catches made in the present study apparently does not reflect the real population density of P. mascittii in Austria. Our personal observation based on sandfly breeding experiences is that P. mascittii is less attracted by light in contrast to other sandfly species. Escaped
sandflies in the laboratory rarely can be trapped in a CDC light trap, but often can be found on apple or peach pieces.

_P. mascittii_ is an assumed vector of _Leishmania_ spp., but data on its natural or experimental transmission capacity are still lacking. Nevertheless, in Germany, _P. mascittii_ was found as the sole sandfly species in areas with at least most probable autochthonous _Leishmania_ infections in humans and animals (Nauke 2002; Nauke et al. 2008a). And, as it is known, that the active dispersal capability of sandflies is very low (e.g. Rioux et al. 1979), it has to be assumed that in these cases, the causative _Leishmania_ strains had been transmitted by _P. mascittii_. Also in Austria, two cases with assumed autochthonous _Leishmania_ infections have been reported (Beyreder 1965; Kollaritsch et al. 1989). Interestingly, these were both from the eastern low parts of Austria, which is the part of Austria, where now sandflies were found and also where in a recent epidemiological study investigating asymptomatic Austrian adults, the highest seropositivity for _Leishmania_ spp. was found (Poeppl et al. 2012).

When speculating on sandfly borne pathogens establishing in Austria, a prerequisite for autochthonous infections is not only the presence of a competent vector, but also of a pathogen reservoir. For _Leishmania_, dogs are certainly the most important reservoir hosts. In Mediterranean countries, _Leishmania_ infections in dogs, and particularly in stray dogs, are very common (e.g. Brandonisio et al. 1992). Infected dogs usually develop conspicuous clinical symptoms leading to a miserable appearance, so that many infected stray dogs have been brought home by compassionate German and Austrian tourists. This has meanwhile even led to such dogs being offered for sale via the internet. According to conservative estimates about 20,000 dogs infected with _Leishmania_ presently live in Germany (Nauke et al. 2008a). In dogs, therapy is usually more complicated than in humans, moreover, dogs are assumed to remain reservoirs even after successful therapy, and also asymptomatic dogs can pass on the infection to sandflies (Alvar et al. 2004, Ashford et al. 1998). Also in Austria, the percentage of infected dogs seems to be high, although until now, the number of dogs that have been investigated for _Leishmania_ infection is still rather low (Edelhofer et al. 1965; Kollaritsch et al. 1989). Interestingly, these were both from the eastern low parts of Austria, which is the part of Austria, where now sandflies were found and also where in a recent epidemiological study investigating asymptomatic Austrian adults, the highest seropositivity for _Leishmania_ spp. was found (Poeppl et al. 2012).

In conclusion, it was shown that sandflies are much more widely distributed in Austria than previously assumed, currently in small foci, but in the course of global warming, further spreading may be expected. This is of potential medical relevance, not only with respect to transmission of _Leishmania_ spp., for which a reservoir is known to exist in Austria, but also of other pathogens, in particular phleboviruses.

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