Insights into the epidemiological link between biting flies and pemphigus foliaceus in southeastern Brazil

Sebastian Vernal, Mateus Pepinelli, Claudio Casanova, Thais M. Goularte, Olivia Kim, Natalia A. De Paula, Mara C. Pinto, Anderson Sá-Nunes, Ana Maria Roselino

Department of Immunology, Institute of Biomedical Sciences, University of São Paulo, São Paulo, Brazil
Division of Dermatology, Department of Clinical Medicine, Ribeirão Preto Medical School, University of São Paulo, Ribeirão Preto, São Paulo, Brazil
Department of Natural History, Royal Ontario Museum, Toronto, Ontario, Canada
Department of Ecology and Evolutionary Biology, University of Toronto, Toronto, Ontario, Canada
Supervintence for Endemic Disease Control, Health Secretat of São Paulo State, Mogi-Guaçu, São Paulo, Brazil
Institute of Biology, Department of Animal Biology, State University of Campinas, Campinas, São Paulo, Brazil
Department of Parasitology, São Paulo State University Julio de Mesquita Filho, Araraquara, São Paulo, Brazil
Department of Immunology, Institute of Biomedical Sciences, University of São Paulo, São Paulo, Brazil

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Background: Black fly and sandfly bites are related to the endemicity of pemphigus foliaceus (PF); however, an immune reaction against the salivary proteins from these flies still requires confirmation in the case of PF patients living in southeastern Brazil.

Purpose: To georeference the distribution of Simuliidae (Diptera: Simuliidae) and Phlebotominae (Diptera: Psychodidae) and of PF cases in the northeastern region of São Paulo State, and to assess the humoral immune response against salivary gland extracts (SGEs) from biting flies in PF patients, relatives, and neighbours.

Methods: PF patients’ medical information recorded between 1965 and 2014 were obtained from the database of the University Hospital. Data on the distribution of fly species were collected from scientific reports and epidemiological databases. Spatial maps relating the distribution of biting flies with PF cases were plotted. Serum IgG antibodies against the SGEs from Simulium nigrimanum, Nyssomyia neivai, and Aedes aegypti (as control) were determined by ELISA.

Results: Two hundred and eighty-five PF cases were distributed in 60 municipalities with a prevalence of 57.5 per million inhabitants, revealing well-defined geographical clusters. S. nigrimanum and N. neivai specimens were registered in eight (13.3%) and 26 (43.3%) of these municipalities, respectively. PF patients, and their relatives presented higher levels of IgG against the SGEs of S. nigrimanum and N. neivai (P < 0.001 for both), but not against the SGE from A. aegypti (P = 0.115 and P = 0.552, respectively), as compared to controls. IgG against the SGEs from S. nigrimanum and N. neivai but not against the SGE from A. aegypti correlated with levels of anti-Desmoglein 1 in PF patients (r = 0.3848, P = 0.039; and r = 0.416, P = 0.022, respectively).

Conclusion: An epidemiological link between biting flies and PF in southeastern Brazil is proposed, implying a possible role of the salivary proteins from these flies in PF etiopathogenesis.

1. Introduction

Pemphigus foliaceus (PF) is an autoimmune bullous disease caused by autoantibodies against desmoglein (DSG) 1. It is subdivided into classic sporadic worldwide Cazenave’s pemphigus and endemic pemphigus (known as Fogo Selvagem in Brazil). Although the pathogenesis of PF remains unclear, genetic and environmental factors have been implicated in the susceptibility to this disease (Abréu-Vélez et al., 2010; Brochado et al., 2016).

Historically, PF cases have been recorded in rural areas of Brazil and other South American countries such as Colombia, Peru, Paraguay, and Venezuela (Abréu-Vélez et al., 2010; Díaz et al., 1989). That insect bites might be associated with PF has been pointed out since 1940 (Vieira, 1940), when an epidemiological link between Simulium nigrimanum (Macquart, 1838) and the endemicity of PF was hypothesized in São Paulo State, southeastern Brazil (Vieira, 1940). PF remains endemic in...
the northeastern region of São Paulo State (Abréu-Vélez et al., 2010; Díaz et al., 1989; Roselino and Almeida, 1995). *S. nigrimanum* bites have been considered a risk factor for the development of PF in an Amerindian reserve in Mato Grosso do Sul State, Brazil (Aoki et al., 2004; Hans-Filho et al., 1996; Eaton et al., 1998; Lombardi et al., 1992). Two other widespread voracious black fly species—*Simulium pertinax* (Kollar, 1882) and *Simulium incrustatum* (Lutz, 1910)—have also been linked to the endemcity of PF in this same reserve (Eaton et al., 1998).

The relation between *S. nigrimanum* and other hematophagous insects with PF has been not explored enough in southeastern Brazil. Higher levels of serum IgG against maxadilan—a highly immunogenic salivary protein described in *Lutzomyia longipalpis* (Lutz and Neiva, 1912), a vector of visceral leishmaniasis (VL) (Lerner et al., 1991), have been determined in PF patients as compared to controls living in a southeastern Brazilian region where PF is endemic (Roselino et al., 2001). There is also evidence that LJM11, another highly immunogenic protein from *Lu. longipalpis*, cross-reacts with antibodies against DSG1 (Qian et al., 2012). Zaraa et al. (Zaraa et al., 2012) have also reported the recognition of the salivary protein from *Phlebotomus papatasi* (Scopoli, 1786) by patients’ sera in a Tunisian region where PF is endemic.

*Nyssomyia intermedia* (Lutz & Neiva, 1912) and *Nyssomyia neivai* (Pinto, 1926) (= syn. *Lutzomyia intermedia*) are the main vectors of *L. (V.) braziliensis* in São Paulo State. Moreover, *N. neivai* has been identified in research collections done systematically in several municipalities of the northeastern region of São Paulo State (Andrade Filho et al., 2007). *Lu. longipalpis* is mainly distributed in the western region of São Paulo State, but it has not been related to VL in the northeastern region of this state (Casanova et al., 2015).

Another important group of biting insects—Culicidae—has never been associated with PF; however, 23 (76%) out of 30 PF patients have reported mosquito bites in a Brazilian Amerindian reserve (Lombardi et al., 1992). Mosquitoes are vectors of various pathogens, including the Dengue and the Zika viruses, and their populations have been also reported in areas of São Paulo State where PF is endemic (Glasser and de Castro Gomes, 2000).

Considering these previous studies, the relation between the distribution of black flies and sandflies with the endemcity of PF in southeastern Brazil requires confirmation. Furthermore, assessment of PF patients exposed to bites of these flies is mandatory to establish an epidemiological link. The present study aims (i) to compare the spatial distribution of black flies (Diptera: Culicidae) and Phlebotominae sandflies (Diptera: Psychodidae) with PF cases georeferenced in the studied region. Considering these previous studies, the relation between the distribution of black flies and sandflies with the endemcity of PF in southeastern Brazil requires confirmation. Furthermore, assessment of PF patients exposed to bites of these flies is mandatory to establish an epidemiological link. The present study aims (i) to compare the spatial distribution of black flies (Diptera: Culicidae) and Phlebotominae sandflies (Diptera: Psychodidae) with PF cases georeferenced in the studied region.

2. Materials and methods

2.1. Mapping and spatial distribution

2.1.1. Type of study

This is an observational study with a retrospective cohort analysis.

2.1.2. PF cases

Medical records of PF cases were collected from the database of the University Hospital of the Ribeirão Preto Medical School, University of São Paulo, Brazil. Records concerned patients who resided in the northeastern region of São Paulo State (hereafter referred as NRSP) at the onset of PF symptoms between 1965 and 2014. The clinical diagnosis of PF was confirmed by acantholytic bullae on the histopathological examination, positive IgG fluorescence around keratinocytes in skin samples, and presence of serum autoantibodies against DSG1 as revealed by ELISA (MBL, Nagoya, Japan).

2.1.3. Study area

NRSP, southeastern Brazil, has a tropical climate and is located between 19°52′ and 22°51′S and 46°16′ and 49°20′W. The region comprises 125 municipalities that cover an area of 51,661 km². This region represents about 21% of the total area of the state and was inhabited by 5,156,660 people in 2016 (IBGE, 2017).

2.1.4. Data on black flies

Reports of *S. nigrimanum* collections in NRSP have been registered since the beginning of the last century (Vieira, 1940). Most data were obtained from Dr Mateus Pepinelli doctoral thesis, in which he reports collecting specimens from 151 streams and rivers across São Paulo State, of which 26 were situated in 25 NRSP municipalities. Collections were accomplished between 2002 and 2005 (Pepinelli, 2008).

2.1.5. Data on sandflies

The distribution of sandflies was obtained from epidemiological data deposited at the database of the Health Secretariat of São Paulo State since 1940, obtained mainly via the VL and American tegumentary leishmaniasis (ATL) surveillance programs. Entomological collections were performed in locations with known or suspected human cases of ATL and in municipalities with suspected or confirmed cases of human and canine VL; a minimum of four dwellings were sampled (Casanova et al., 2015; Casanova et al., 2014; Secretary of Health of São Paulo State, 2011; Secretary of Health of São Paulo State, 2008).

2.1.6. Spatial maps

The scale for map construction was 1:3,266,000 (0–130.7 latitude/longitude projection). Cases were plotted against the number of patients in each municipality of NRSP. Black fly and sandfly records were also plotted per municipality.

2.1.7. Nomenclature


2.2. Assessment of exposure to bites

2.2.1. Type of study

This is a transversal study.

2.2.2. Samples of insects

Given their pest status and wide distribution, *S. nigrimanum* and *N. neivai* were included as the most representative simulid and phlebotome, respectively, of the target region. *A. aegypti*—another biting fly that is widely distributed in NRSP (Glasser and de Castro Gomes, 2000) but which has not been related to PF—was included as a control.

2.2.2.1. *Simulium nigrimanum*. Adult females were collected in Ribeirão Corrente river, in the municipality of Jardimópolis, NRSP (20°30′30.04″S, 47°33′04.34″W) during oviposition, while flying, or while biting humans. Aquatic life stages were collected for additional identification, following Hamada & Pepinelli methods (Hamada and Pepinelli, 2004).

2.2.2.2. *Nyssomyia neivai*. Adult female samples were obtained from a laboratorial colony supervised by Dr Mara Pinto, State University of São Paulo, Araraquara, Brazil.

2.2.2.3. *Aedes aegypti*. Adult female samples were obtained from a laboratorial colony supervised by Dr Anderson Sá-Nunes, Institute of Biomedical Sciences, University of São Paulo, São Paulo, Brazil.
2.2.3. Salivary gland extracts (SGEs)

Salivary glands were dissected and stored in phosphate-buffered saline (PBS, pH 7.4). Salivary protein extracts were properly characterized in SDS gel (Supplementary Fig. 1S). Immediately before use, the SGEs were disrupted by ultrasonication and centrifuged at 14,000 g; the resulting supernatant—SGE—was used in this study (Bizzarro et al., 2013; Souza et al., 2010).

2.2.4. In-house ELISA

To assess exposure to bites, an in-house ELISA assay was standardized with the SGE from each species of insect. Briefly, 300 ng of SGE diluted in PBS was incubated in 96-well microplates (Corning, Sigma-Aldrich, St. Louis, USA) overnight and blocked with PBS/Tween/0.005%/BSA 1% for an hour. After three washings, the patients’ sera (1:100, in duplicate) were monitored with secondary antibody 1:10,000 (anti-human IgG, Abcam, Cambridge, USA). Then, the wells were washed four times, and 100 μL of chromogenic substrate (3, 3′,5 ,5′-Tetramethylbenzidine-TMB) was added. After 15-min incubation at room temperature (in the dark), 50 μL of stop solution (0.2 M H2SO4,) was added. The Optical Density (OD) values were determined with an ELISA plate reader (Biochrom Asys Expert Plus Microplate Reader, Cambourne, Cambridge, UK) fitted with a 450-nm absorbance filter. In-house ELISA was performed by a blinded technician, in duplicate, on the same day; the same reagents were used to avoid plate variability. The experiments were performed twice. In-house ELISA was not considered as a diagnostic tool for PF. Hence, instead of positive/negative results being presented, in-house ELISA was used only as a comparative tool to assess the humoral response to SGEs. In-house ELISA was validated by using serum samples from female rabbits before and after immunization with each SGEs (Supplementary Material and Methods).

2.2.5. Serum samples

Thirty serum samples from PF patients were compiled randomly from the Pemphigus Bank of the Laboratory of Dermatology at the University Hospital of the Ribeirão Preto Medical School, University of São Paulo, Brazil. All the patients presented active clinical disease with the presence of Nikolsky’s sign at the moment serum was collected. The control groups consisted of 30 healthy relatives of PF patients and 30 healthy neighbours of PF patients’ living in the same studied region and without any clinical manifestation of PF. They comprised first-degree family members and accompanying neighbour on the occasion of the outpatient appointment.

2.2.6. Statistical analysis

Parametric model assumptions were assessed by means of a normal plot. Demographic and clinical data from patients and controls were compared by non-parametric tests as appropriate. Kruskall–Wallis followed by Dunn’s test was employed to compare ELISA OD values amongst the groups. The Spearman’s test was used to correlate the levels of antibodies against SGEs and anti-DSG1 as well as the levels of antibodies and time of disease in the PF group. Significance was set at α = 5%. GraphPad Prism 7 was used for the statistical analysis (GraphPad software Inc., La Jolla, CA, USA).

2.2.7. Ethics

This study was approved by the local Human Research Ethics Committee (#866.027/2014), in accordance with the ethical standards of the Helsinki Declaration of the World Medical Association; it was also approved by the Animal Research Ethics Committee (#78/2015).

3. Results

3.1. Georeferencing analysis

The georeferenced maps illustrate the distribution of 285 PF cases in 60 (48%) out of 125 municipalities whose prevalence comprised 57.5/1,000,000 inhabitants in NRSP. Six municipalities concentrated ten or more PF cases (Fig. 1).

Concerning the distribution of anthropophilic Simuliidae species, the compiled data refer to specimens collected in 25 (20%) out of 125
municipalities. Fig. 2A-C shows that Simuliidae was distributed around the main PF focus. *S. nigrimanum* was recorded in 11 (44%) out of 25 municipalities (Fig. 2A), including eight (72.7%) municipalities where PF cases were registered. One of these municipalities corresponded to a well-defined PF geographical cluster (municipality of Franca, number 2, Fig. 1). *S. pertinax* was distributed in nine (36%) out of 25 municipalities; PF cases occurred in five (55.5%) of them (Fig. 2B). *S. incrustatum* was detected in 12 (48%) out of 25 municipalities; PF cases were registered in eight (66.6%) of them (Fig. 2C). It is noteworthy that *S. nigrimanum*, *S. pertinax* and *S. incrustatum* have been found inhabiting municipalities. Fig. 2A-C shows that Simuliidae was distributed around the main PF focus. *S. nigrimanum* was recorded in 11 (44%) out of 25 municipalities (Fig. 2A), including eight (72.7%) municipalities where PF cases were registered. One of these municipalities corresponded to a well-defined PF geographical cluster (municipality of Franca, number 2, Fig. 1). *S. pertinax* was distributed in nine (36%) out of 25 municipalities; PF cases occurred in five (55.5%) of them (Fig. 2B). *S. incrustatum* was detected in 12 (48%) out of 25 municipalities; PF cases were registered in eight (66.6%) of them (Fig. 2C). It is noteworthy that *S. nigrimanum*, *S. pertinax* and *S. incrustatum* have been found inhabiting
streams of the same municipality area in three cases, and that PF was reported in two of these municipalities (Cajuru and São Carlos municipalities).

Regarding the distribution of sandflies (Fig. 2D-I), the compiled data refer to specimens collected in 76 (60.8%) out of 125 municipalities. N. neivai was recorded in 55 (44%) municipalities (Fig. 2D). PF cases occurred in 26 (47.2%) out of these 55 municipalities. The presence of N. neivai in four out of six well-defined PF clusters is worthy of note. Comparison of the spatial distribution of N. neivai with the spatial distribution of other studied sandflies revealed that this species was the most widely distributed in NRSP, followed by Nyssomyia whitmani (Antunes & Coutinho, 1939) (Fig. 2E), reported in 33 (26.4%) out of 125 municipalities, 13 (39.4%) of which had reported PF cases.

Lu. longipalpis was investigated in 44 (35.2%) out of 125 municipalities. There were positive records in nine (20.5%) out of 44 municipalities, which represented less than 8% of the total number of municipalities in the region. The distribution of Lu. longipalpis did not match the occurrence of any well-defined PF cluster (Fig. 2F).

We also plotted the distribution of other sandfly species—Pintomyia pessoai (Coutinho & Barreto, 1940), Pintomyia fischeri (Pinto, 1926), and Migonemysia migonei (França, 1920) (Fig. 2G-I).

3.2. Immunological study

We tested serum samples obtained from 30 PF patients (median age = 47 years, 18 females), 30 PF patients’ healthy first-degree relatives (median age = 46 years, 23 females), and 30 PF patients’ healthy neighbours (median age = 55 years, 12 females) as controls living in the same area where PF is endemic. Table 1 summarizes the clinical and the laboratorial data (for more details please check Supplementary Table 1S and Supplementary Table 2S). No significant differences were found among groups related to gender (P = 0.117). There were no significant differences between PF patients as compared to relatives and neighbours in terms of gender, but healthy neighbours were older than healthy relatives (P = 0.029). The median time of PF evolution was nine years (p25th 2 years, p75th 18 years), and most of the patients (83.3%) were being treated for PF at the moment serum was collected. The PF patients that had not started immunosuppressive treatment or who had been without treatment for at least 60 days at the moment serum was collected tended to have higher values of anti-DSG1 antibodies against the SGEs from S. nigrimanum and N. neivai as compared to PF patients under treatment (P = 0.065) (data not shown).

Fig. 3 shows the detection of IgG against the SGE from each insect. There were no statistical differences amongst the PF patients, their healthy relatives, and their neighbours for the SGE from A. aegypti (P = 0.320). On the other hand, PF patients presented higher levels of IgG antibodies against the SGEs from S. nigrimanum and N. neivai as compared to PF patients’ relatives (P < 0.001 for both SGEs), but not as compared to PF patients’ neighbours (P = 0.115 and P = 0.552, respectively). Higher levels of antibodies against the SGEs from S. nigrimanum and N. neivai were also found in PF patients’ relatives as compared to PF patients’ neighbours (P < 0.001). Levels of IgG against the SGEs from S. nigrimanum and N. neivai but not against the SGE from A. aegypti were significantly higher in PF patients who had not started immunosuppressive treatment or without treatment for at least 60 days as compared to patients under treatment (P = 0.021, P = 0.19, and P = 0.327, respectively) (data not shown).

Levels of anti-DSG1 antibodies correlated positively with antibodies against the SGEs from S. nigrimanum and N. neivai (r = 0.3277, P < 0.001; and r = 0.3958, P < 0.001, respectively), but not with antibodies against the SGE from A. aegypti (r = −0.1515, P = 0.154) (Fig. 4A). Antibodies against the SGEs from S. nigrimanum and N. neivai also correlated positively with anti-DSG1 in PF patients (r = 0.3848, P = 0.039; and r = 0.416, P = 0.022 respectively) (Fig. 4B). There was no correlation between the levels of antibodies against DSG1 and the SGE from each insect with the duration of the disease in PF patients (P > 0.05 in all cases) (data not shown).

4. Discussion

The expansion of urban boundaries has been accompanied by increased occurrence of diseases (e.g., leishmaniasis) that had been previously restricted to rural areas (Tolezano, 1994), a phenomenon that seems to apply for our study. Population growth associated with expansion of agricultural production has resulted in urban invasion of forested regions. This scenario represents a new stage of human cohabitation with the local fauna, which had been previously confined to places outside the cities (Tolezano, 1994). Historically, PF was distributed in rural areas and in areas close to rivers, which reinforces the hypothesis that Simuliidae and/or Phlebotominae might be associated with PF (Abrêu-Vélez et al., 2010; Aoki et al., 2004; Chiossi and Roselino, 2001; Diaz et al., 1989; Eaton et al., 1998; Roselino and Almeida, 1995).

The present study shows coincidental georeferenced maps of anthropophilic Simuliidae and Phlebotominae species and PF cases and proposes an epidemiological link between them (Abrêu-Vélez et al., 2010; Chiossi and Roselino, 2001; Diaz et al., 1989; Roselino and Almeida, 1995). Spatial geographical distribution maps of Simuliidae, Phlebotominae, and PF cases overlap 34 municipalities. The epidemiological link between S. nigrimanum and PF, described over 70 years ago in the municipality of Franca (Vieira, 1940), a well-defined focus of PF characterized as number 2 in Fig. 1, represents the first attempt to establish a relationship between Simuliidae and PF. The present study confirms that PF is still prevalent in Franca, as well as anthropophilic Simuliidae species. Unfortunately, we have not been able to collect Simuliidae species in the other well-defined focus of PF (municipalities numbered 1 and 3–6 in Fig. 1). However, the surrounding cities surveyed present populations of anthropophilic black fly species reported herein, so it is expected that S. nigrimanum populations inhabit streams and rivers in some of these municipalities.

Although S. nigrimanum has been more frequently associated with PF, other anthropophilic species like S. pertinax and S. incrustatum are widely distributed in regions with reported PF cases. Previous studies have also mentioned that S. pertinax and S. incrustatum are present in the Limão Verde indigenous reserve, a region where Brazilian PF is significantly endemic (Eaton et al., 1998).

Simuliidae species have been related to PF on the basis of two main hypotheses: (i) black fly species could carry a virus, or (ii) salivary proteins may mimic DSG (Abrêu-Vélez et al., 2010; Aoki et al., 2004; Chiossi and Roselino, 2001; Roselino and Almeida, 1995). Ribeiro et al.
have recently compared the peptide sequence of the *S. nigrimanum* sialotranscriptome with the peptide sequence of DSG to identify some candidate proteins that could induce anti-DSG cross-reactivity (Ribeiro et al., 2010). Although these authors found some similarity, no cross-reactivity with salivary peptide has been proven so far. The sialotranscriptome analysis of other Simuliidae species is desirable because salivary proteins shared by the three species identified in NRSP could be recognized. Moreover, this analysis could uncover a putative black fly salivary antigenic mimicry with DSG. The positive correlation between anti-*S. nigrimanum* SGE with anti-DSG1 in PF patients suggests...
a possible cross-reactivity between antigens; notwithstanding, further studies are necessary to prove this possible mimicry and to find the precise salivary peptide that is involved in the process.

The involvement of the sandfly \textit{Lu. longipalpis} in PF has also been studied. This sandfly is an important vector of \textit{Leishmania infantum} chagasi (Chagas & Cunha, 1937), a parasite that causes VL in Brazil. Many reports have described the transmission of VL in some regions of São Paulo State (Casanova et al., 2015; Secretary of Health of Sao Paulo State, 2011). In the NRSP municipalities, \textit{Lu. longipalpis} occurred without association with PF clusters (Fig. 2F). Roselino et al. (2001) (Roselino et al., 2001) have shown that sera collected from PF patients exhibit higher levels of IgG antibodies against maxadilan—a salivary protein from \textit{Lu. Longipalpis}—as compared to healthy controls living in the same endemic area. There is also evidence that the LJMM11 salivary peptide from \textit{Lu. longipalpis} cross-reacts with antibodies against DSG1 (Qian et al., 2012). Furthermore, IgE antibodies against LJMM11 have been proposed to trigger PF in some Amerindian populations living in endemic regions (Qian et al., 2015).

Nevertheless, \textit{Lu. longipalpis} is poorly distributed in the NRSP. Establishing an epidemiological link between LJMM11 and PF in the NRSP would be ground-breaking. For this purpose, a LJMM11-simile salivary peptide could be expressed in other biting fly species that inhabit NRSP, such as \textit{N. neivai}, a vector of AT in the studied region (Casanova et al., 2015). Recently, Ribeiro and our group have described the \textit{N. neivai} saliatranscriptome (data not published – available in GenBank NCBI-NIH, USA (term: \textit{Nyssomyia neivai})), and surprisingly we did not find a LJMM11-simile peptide, but 16 putative sequences of maxadilan-simile were described for \textit{N. neivai}.

On the basis of the production of antibodies against the SGEs from \textit{S. nigrimanum} and \textit{N. neivai} but not against the SGE from \textit{A. aegypti}, we have demonstrated that PF patients, PF patients’ healthy relatives, and PF patients’ neighbours living in the same area were exposed to bites by these flies, which has helped us to establish the epidemiological link. Remarkably, higher levels of IgG antibodies against the SGEs from \textit{S. nigrimanum} and \textit{N. neivai} but not against the SGE from \textit{A. aegypti} have been detected in PF patients as compared to PF patients’ neighbours. These results have led us to hypothesize that specific salivary gland proteins from black flies and sandflies, but not from mosquitoes possibly participate in PF. To our knowledge, no other study has dealt with exposure to \textit{S. nigrimanum} bite or with the humoral response against its SGE.

The increased humoral response against the SGEs from \textit{S. nigrimanum} and \textit{N. neivai} in PF patients’ relatives deserves attention. Genetic factors could be involved in this humoral response. In fact, three out of 30 PF patients’ relatives but no PF patients’ neighbours presented positive anti-DGG1 or antibodies against the SGEs from \textit{S. nigrimanum} and \textit{N. neivai} (data not shown); however, none of them have developed clinical PF yet. Further determination of the HLA susceptibility/protection profile of PF patients’ relatives is necessary to compare it to the HLA profile of PF patients (Brochado et al., 2016).

Finally, it is important to discuss the positive correlation between the antibodies against the SGEs from \textit{S. nigrimanum} and \textit{N. neivai} in the three groups (PF patients; PF patients’ relatives, PF patients’ neighbours). This correlation shows that exposure to the bites of both species is possible because they are similarly distributed in NRSP. Nevertheless, the hypothesis of cross-reactivity between their salivary gland peptides should be carefully analysed. Although the two incriminated species are not closely related (Wiegmann et al., 2011), similar salivary proteins with similar antigen effects might be playing a role on PF pathogenesis

4.1. Limitations of the study

Because the data reported here have been compiled from a third-level hospital, the number of PF cases could be underestimated due to lack of notification of pemphigus in Brazil and to possible treatment of patients at secondary healthcare level. Hence, we believe that the prevalence of PF in southeastern Brazil could be even higher.

Fig. 2 is a representative distribution of nine species of flies in NRSP; however, their distribution is obviously dynamic and might change over time. Ecological, epidemiological, and demographic transformations in NRSP could determine variations on the distribution of the different studied species in this region. Regarding the aim of this study, maps have been plotted for pedagogical and research purposes regarding PF cases for further selection of the most representative black fly and sandfly species in NRSP. Further ecological studies may provide fly population variations over the years.

No immunosuppressed PF patients presented higher levels of antibodies against the SGEs from \textit{S. nigrimanum} and \textit{N. neivai} but a tendency to have higher levels of anti-DGG1 as compared to patients under treatment. Therefore, immunosuppression treatment of PF patients could be underestimating the levels of anti-SGE IgG levels because the levels of anti-DGG1 decreased upon immunosuppression. Repeating the assays with PF patients that have never received any treatment is desirable.

5. Conclusion

PF cases and the spatial distribution of \textit{S. nigrimanum} and \textit{N. neivai} biting flies have shown defined geographical clusters in NRSP. Increased levels of IgG antibodies against the SGEs from \textit{S. nigrimanum} and \textit{N. neivai} in PF patients confirm exposure to fly bites, suggesting an epidemiological link between flies and PF in southeastern Brazil.

Summary

An epidemiological link between biting flies and Pemphigus Foliaceus (PF) in Brazil is proposed, implying a possible role of biting fly salivary gland proteins in PF etiopathogenesis.

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Conflict of interest

The authors have no conflicts of interest to declare.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.actatropica.2017.09.015.