



Laboratory animal allergy reduction from 2001 to 2016: An intervention study

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ABSTRACT

Exposure to laboratory animals (LA) can cause allergic sensitization and symptoms as rhinitis, conjunctivitis, asthma, anaphylaxis and dermatitis. In 2000, a program was instituted at Trieste Universities to decrease LA allergy among scientists and technicians working with animals. The aim of our study was to investigate LA allergy in workers exposed to LA from 2001 to 2016, and to verify the effects of a preventive program.

Four hundred sixty seven people underwent pre-employment screening for a job with laboratory animals at Universities of Trieste consisting in a medical examination, a full respiratory and allergy anamnesis, using a standardized questionnaire, skin prick test with common and occupational allergens, and spirometry. Every year, each worker repeated the medical examination and underwent again tests and questionnaire. Each worker can ask for a medical examination and skin prick test, in case of onset of symptoms. Logistic multivariate analysis and generalized equation estimation were used, to verify factors associated to LA allergy.

Sensitization to LA decreased in years, going from 25.6% in 2001–2004 to 8.2% in 2013–2016 ($p < 0.001$). Multivariate logistic regression analysis confirmed the role of atopy by prick test ($OR = 6$; $IC95\% 2.2$ – 16.6), of common allergic symptoms ($OR = 2.9$; $IC95\% 1.4$ – 6.39) and of calendar periods. No association was found between LA allergy, years, and hours of exposure. Our study demonstrated a significant reduction of LA allergy after the application of a preventive program.

1. Introduction

Exposure to laboratory animals (LA) can cause allergic sensitization and symptoms. Prevalence estimation of LA allergy ranged between 11 and 44% of workers, with higher figures in old reports and lower prevalence in more recent studies [1–5]. Symptoms included allergic rhinitis, conjunctivitis, asthma, anaphylaxis, and skin symptoms [4–6]. Chest and skin symptoms were associated with increasing intensity to exposure to rat urinary allergy [7] and recent studies [8] demonstrated an increased risk for exposed people to develop symptoms compared to non-exposed, but sensitized to common allergens controls. Occupational asthma decreased from old studies in the 70's to more recent ones, but a further reduction of exposure is needed to prevent the onset of occupational rhinitis [4]. A previous study of our group [9] found that LA allergy ranged from 11.8 to 14.8% according to work seniority, with 5% and 7.9% of subjects that reported work related asthma and rhinitis, respectively. Our figures were low compared with other reports in Brazil [8], in Korea [10] and in USA [11]. Personal atopy and total IgE level are risk factors for the sensitization to LA [3,6,10] and incidence of symptoms is significantly related to exposure in a dose-related manner for some authors [6,7].

LA allergy remains a significant problem for LA workers despite the interventions done to reduce exposure through a better local exhaustion system during animal handling, procedural and personal controls with health surveillance system for workers, and the compulsory use of a protective personal equipment. In 2000, a program was instituted at Trieste Universities to decrease LA allergy among scientists and technicians working with animals. The program included education and training, institution of good work practice to reduce exposure to LA, the use of a local exhaustion system during all working procedures, the use of protective respiratory protection, and a medical surveillance program with symptoms assessments, skin prick test for common and occupational allergens including latex (as researchers can wear latex gloves) and animal danders. Work practice modification included the institution of wet cleaning practices, and wet-shaving practices (i.e. using water or a foam shaver), the avoidance for researchers to go into stables, and the use of local exhaustion system during the work with animals. Environmental controls were implemented including high efficiency particulate air-filtered room ventilation and use of dust-free bedding [12]. All workers had to wear a mask with high efficiency (PP3) to limit the inhalation of allergens.

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The medical surveillance assessment with skin prick test and spirometry were performed before the start of the work with animals and every year or before, if occupational symptoms occurred during the course of their job. Educational training to increase knowledge on LA was undergone by every worker, with the aim to recognize mild symptoms occurring and ask for medical control.

The aim of the study was to evaluate LA allergy in workers exposed to LA from 2001 to 2016, and to verify the effects of a preventive program.

2. Methods

Between 2001 and 2016, 467 people underwent pre-employment screening for a job with laboratory animals at University of Trieste and SISSA (Scuola Internazionale Superiore di Studi Avanzati). Pre-employment screening was done by the Unit of Occupational Medicine at University of Trieste, and consisted in a medical examination, a full respiratory and allergy anamnesis using a standardized questionnaire [9,13], skin prick test with common and occupational allergens and spirometry. Every year each exposed worker repeated the medical examination and underwent skin prick test for LA and questionnaire. Each worker asked for a new medical examination, and were skin prick tested again, in case of onset of symptoms. At baseline, some workers resulted already exposed to laboratory animals, during thesis or training period or in a previous job in other research facilities. The study was inserted in a compulsory program for prevention of occupational diseases following the Italian Law 81/2008, local Ethics Committee was informed about the retrospective analysis performed. Written informed consent was obtained from all subjects after reading and discussing the protocol individually. All workers underwent lung function evaluation using a Biomedin Spiromether (Padua, Italy) with the evaluation of forced expiratory volume in 1 s (FEV1) and vital capacity (VC), Forced Expiratory Flow 25–75 (FEF25–75), according to the American Thoracic Society recommendations [14]. As all values were normal, results are not reported.

2.1. Skin prick test

Skin prick test (SPT) was performed according to the recommendations of the European Academy of Allergology and Clinical Immunology [15], using a panel of common allergens (*Dermatophagoides farinae* and *Pteronyssinus*, cat and dog, *Alternaria*, pollen of *Graminae*, *Betula*, *Corylus*, *Oleaea*, *Parietaria* spp) and occupational allergens included latex, rat, mouse, rabbit, guinea pig, hamster extracts (Lofarma Milano, Italy). Histamine dihydrochloride (10 mg/ml) served as positive and the solvent as negative control. A wheel of ≥ 3 mm in diameter was considered as positive reaction. Atopy was defined when a subject was positive to at least one common allergen.

2.2. Questionnaire

The questionnaire used contained questions on personal (nose, skin and lower respiratory tract symptoms) and familiar allergic diseases, allergic drug intake, smoking habits, exposure to laboratory animals and symptoms in connection with this exposure, usage of safety equipment [9,13]. Allergic symptoms due to working with laboratory animals were defined as the presence of allergy during working hours or after contact with laboratory animals. Allergic symptoms were divided into 4 groups: nasal symptoms defined as sneezing or running nose with production of nasal secretions; eye symptoms, defined as itching or smarting eyes; asthma, defined as the presence of shortness of breath and wheezing; cough, defined as cough without secretions, and skin symptoms, defined as itching and red skin. The sensitization period was defined as the period between the first exposure to laboratory animals and the first occurrence of symptoms of laboratory animal allergy.

2.3. Exposure evaluation

As a surrogate for exposure intensity the mean numbers of hours per month a person was exposed to laboratory animals was used. Four calendar periods, for those entering the cohort, were distinguished with baseline data: 2001–2004, 2005–2008, 2009–2012, 2013–2016. Four periods of exposures were considered: < 1 year; 1 year; 2–3 years, > 3 years.

2.4. Statistical analysis

Statistical analysis was performed using STATA, Inc. (Texas, USA). Comparison between never exposed and exposed workers was done using chi-square technique for categorical data and by *t*-test for age, seniority of work and exposure data. Association between sensitization to laboratory animals, calendar periods, intensity of exposure and related factors were performed using the univariate and multivariate regression analysis. Results are reported as Odds ratio and 95% Confidence intervals. Factors involved in LA allergy during the follow-up were analysed using the Generalized Equation Estimation (GEE). A *p* value < 0.05 was settled as significant.

3. Results

467 subjects exposed to LA were involved in the study. They represent the 98% of exposed subjects. 145 were never exposed to LA and 322 were already exposed for 4.1 ± 6.2 (95% CI 2.9–5.4) years in men and 3.1 ± 6.9 (95% CI 2.1–4.1) years in women. Hours of exposure to LA ranged from 25.4 to 50.8 per month (95% CI). The Table 1 reports the characteristics of the two groups. The group of “never exposed” resulted significantly younger than “already exposed”, as expected, and were represented by students in the 36.5 and 42.7% of cases, in men and women respectively. Women reported to have a higher familiarity for atopy, while men resulted more atopic by prick test (55.6% and 60.1% in men never and already exposed, respectively). Percentage of allergic symptoms was similar between the two groups, but asthma was more prevalent in exposed vs never exposed (12% vs 9.6%), without reaching the statistical significance. LA sensitization resulted significantly higher in already exposed subjects with 14.4% and 13.7% vs 7.9% and 4.9% in never exposed, in men and women, respectively (*p* < 0.001). LA allergy involved mainly mouse (11% sensitization in men and 5.9% in women), followed by rat (8.5% sensitization in men and 4.9% in women), rabbit, guinea pig and hamster. Prevalence of latex allergy was low with small differences in groups (0.85–4.9%) without reaching the statistical significance. More than half of subjects used latex gloves during work but gloves were without powder and at low latex release, to minimize the risk of sensitization.

Symptoms in subjects sensitized to LA are reported in Table 2. The 9 workers, never exposed to LA but sensitized to them are all atopic to common allergens and 2 of them reported perennial rhinitis. Thirty subjects (67%) already exposed, and sensitized to LA reported symptoms, 20 had rhinitis (6.2% of exposed), 14 asthma (4.3% of exposed), 5 dermatitis and 2 urticaria. Table 3 reports the univariate logistic regression analysis of factors involved in LA sensitization in never and already exposed to LA. Atopy by prick test was strongly associated to LA sensitization (OR = 9.44; IC95% 3.6–24.6) as well as common allergic symptoms (OR = 4.4; IC95% 2.3–8.4), asthma (OR = 5.9; IC95% 2.9–12.00) and symptoms during work (OR 2.5; IC95% 1.3–9.4). Sensitization to LA decreased in years (Fig. 1) going from 25.6% in 2001–2004 to 8.2 in 2013–2016, with higher percentages in females, but with a significant reduction during time: OR = 0.20; IC95% 0.07–0.53 in subjects evaluated in 2009–2012 and OR = 0.37; IC95% in those evaluated in 2013–2016, compared to those exposed in 2001–2004. No association was found between years and hours of exposure. Multivariate logistic regression analysis confirmed the role of atopy by prick test (OR = 6; IC95% 2.2–16.6), of common allergic

Table 1
Characteristics of the population analysed at the first control.

	Never exposed		Already exposed	
	Male (N. 63)	Female (N. 82)	Male (N. 118)	Female (N. 204)
Mean age years $\pm$ SD	28.9 $\pm$ 7.7	26.3 $\pm$ 4.5	31.0 $\pm$ 8.7 [*]	28.3 $\pm$ 5.7 [*]
Hours per month exposed to LA hours $\pm$ SD (CI 95%)	0	0	40.9 $\pm$ 53.9 (31–50.8)	32.0 $\pm$ 47.0 (25.4–38.5)
Years exposed to LA years $\pm$ SD (CI 95%)	0	0	4.1 $\pm$ 6.2 (2.9–5.4)	3.1 $\pm$ 6.9 (2.1–4.1)
Smokers N. (%)	19 (30.1)	18 (21.9)	45 (38.1)	53 (26.0)
Students N. (%)	23 (36.5)	35 (42.7)	21 (17.8)	62 (30.4)
PhD/Post doc N. (%)	27 (42.9)	38 (46.3)	41 (34.7)	44 (21.6)
Researcher/Professors/Technicians	13 (20.4)	9 (11.0)	41 (35.7)	44 (21.6)
Current smokers N. (%)	19 (30.2)	18 (21.9)	45 (38.1)	53 (26.0)
Latex gloves use N. (%)	23 (51.3)	38 (59.1)	92 (77.9)	174 (85.3)
Pet owners N. (%)	22 (44.9)	38 (58.5)	92 (79.3)	73 (36.3)
Familiar atopy N. (%)	18 (28.6)	37 (45.1) [*]	34 (29.0)	88 (43.3) [*]
Atopy by prick test N. (%)	35 (55.6) [*]	31 (37.8)	71 (60.1) [*]	96 (47.1)
Common allergic symptoms N. (%)	20 (31.7)	29 (35.4)	35 (29.7)	57 (28.0)
Pollinosis N. (%)	19 (30.1)	20 (24.4)	29 (24.6)	47 (23.0)
Oculorhinitis N. (%)	20 (30.1)	28 (34.1)	35 (29.7)	57 (28.0)
Asthma N. (%)	6 (9.6)	8 (9.8)	14 (12.0)	23 (11.7)
Cough N. (%)	7 (11.1)	12 (14.7)	21 (17.8)	42 (20.7)
Urticaria N. (%)	0	3 (3.7)	2 (1.7)	0
Dermatitis N. (%)	3 (4.7)	4 (4.9)	3 (2.5)	8 (3.9)
Skin prick tests +				
LA sensitization N. (%)	5 (7.9)	4 (4.9)	17 (14.4) [*]	28 (13.7) [*]
Dog N. (%)	18 (28.6) [*]	9 (10.9)	34 (28.8) [*]	38 (18.6)
Cat N. (%)	12 (19.0) [*]	5 (6.1)	22 (18.6) [*]	19 (9.3)
Professional skin prick test +				
Mouse N. (%)	2 (3.2)	2 (2.4)	13 (11) ^{*,*}	12 (5.9)
Rat N. (%)	0	0	10 (8.5)	10 (4.9)
Guinea pig N. (%)	0	1 (1.2)	4 (4.3)	3 (1.5)
Rabbit N. (%)	2 (3.2)	1 (1.2)	4 (4.3)	10 (4.9)
Hamster N. (%)	1 (1.6)	0	4 (4.3)	3 (1.5)
Latex N. (%)	1 (1.6)	4 (4.9)	1 (0.85)	3 (1.5)

*P < 0.05.

Table 2
Characteristics of subjects sensitized to LA.

	Never exposed N. 9	Exposed N. 45
Female N. (%)	4 (44)	28 (62)
Atopy (by prick test) N. (%)	9 (100)	40 (88.9)
Without symptoms N. (%)	7 (79)	15 (33)
With symptoms	2 (21)	30 (67)
-Rhinitis n. (%)	2 (100)	20 (67)
-Asthma n. (%)	0	14 (47)
-Dermatitis n. (%)	0	5 (17)
-Urticaria n. (%)	0	2 (6.7)

symptoms (OR = 2.9; IC95% 1.4–6.39) and of calendar periods (OR = 0.31; IC95% 0.11–0.92; OR = 0.19; IC95% 0.06–0.59; OR = 0.31; IC95% 0.1–1) for subjects studied in 2005–2009, 2009–2019, 2013–2006 compared to those studied in 2001–2004, respectively (see Table 4).

Seventy-five subjects were studied in follow-up for 3.84 ± 1.15 years and their data are reported in Table 5. LA allergy was found in 9.3% of subject at the first examination and in 14.7% in the last examination, mainly related to rat and mouse sensitization. Table 6 analysed factors involved in LA sensitization during follow-up using the Generalized Estimation Equations. The more important factors were common allergic symptoms (OR = 10.9; IC95% 2.54–46.9) and atopy (OR = 8.1; IC95% 1.7–37.4) while no association was found with seniority of work and hours of exposure. A protective effect was associated to examinations in 2013–2016 compared to those done in 2001–2004. Table 7 analyses subjects that resulted sensitized to LA in the first and the last examinations. At the start of the study 6 subjects were already sensitized to LA, all

Table 3
Univariate logistic regression analysis of factors involved in LA sensitization.

Factors	Never exposed N. 145 OR (CI 95%)	Exposed N. 322 OR (CI95%)
Female	0.59 (0.15–2.3)	0.95 (0.49–1.8)
Age	0.94 (0.80–1.1)	1.0 (0.95–1.04)
Smokers	1.00 (0.80–2.7)	1.5 (0.73–3.1)
Atopy (by prick test)	All atopics	9.44 (3.6–24.6)
Familiar atopy	2.1 (0.54–8.2)	1.3 (0.7–2.55)
Common allergic symptoms	4.7 (1.04–18.3)	4.4 (2.3–8.4)
Common Asthma	4.4 (0.98–19.5)	5.9 (2.9–12.0)
Symptoms during work	–	2.5 (1.3–4.9)
Pet owner	1.2 (0.26–5.8)	0.8 (0.4–1.6)
Cat SPT +	4.4 (0.98–19.5)	3.1 (1.44–6.7)
Dog SPT +	20.4 (4.0–105)	4.9 (2.5–9.4)
Calendar period:		
-2001–2004		1
-2005–2008		0.44 (0.18–1.13)
-2009–2012		0.20 (0.07–0.53)
-2013–2016		0.37 (0.13–1.00)
Seniority of work (years)		1.0 (0.97–1.10)
Hours of exposure/week		1.0 (0.99–1.00)

were atopic to common allergens and 3 were already exposed. All reported symptoms during work and outside the work, but their symptoms did not increase during the follow-up. The two asymptomatic subjects did not develop symptoms during the follow-up and their skin prick test were negative in the last control. During the follow-up we had 7 incidence cases, 4 with symptoms (3 with rhinitis, 3 with cough and 1 with conjunctivitis) and 3 without symptoms. Their details are reported [supplemental material Table 1](#). Rat and mouse were the allergens more frequently positive.

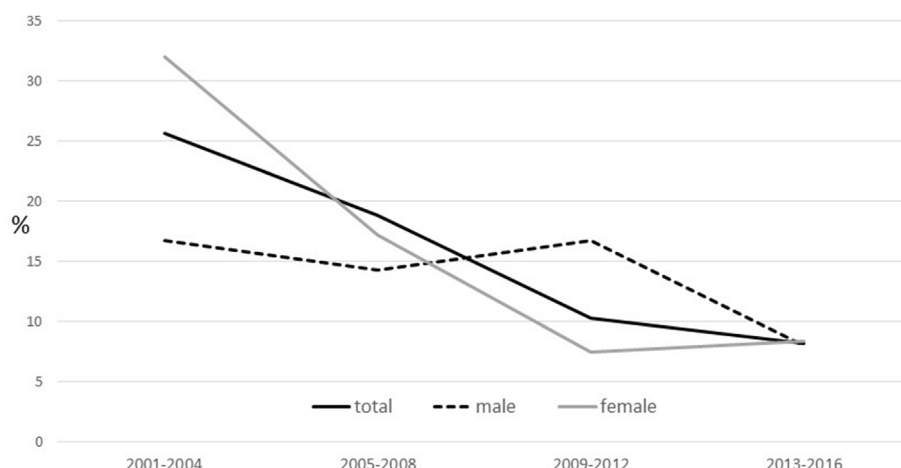


Fig. 1. Sensitization trend to Laboratory animals (%) in the population studied.

Table 4
Multivariate logistic regression analysis of factors involved in LA sensitization.

Factors	Never exposed N. 145 OR (CI 95%)	Exposed N. 322 – Model 1 OR (CI95%)	Exposed N. 322 – Model 2 OR (CI95%)
Female	1.21 (0.26–5.7)	1.18 (0.58–2.4)	1.2 (0.58–2.5)
Age	0.94 (0.81–1.1)	1.0 (0.96–1.01)	0.96 (0.96–1.1)
Atopy (by prick test)	-	7.1 (2.6–19.3)	6.00 (2.2–16.6)
Common allergic symptoms	1.1 (0.2–6.2)	2.3 (1.15–4.7)	2.9 (1.4–6.3)
Asthma	-	-	-
Dog SPT +	21.3 (3.1–147)	-	-
Calendar period:			
-2001–2004		1	1
-2005–2008		0.44 (0.18–1.13)	0.31 (0.11–0.92)
-2009–2012		0.20 (0.07–0.53)	0.19 (0.06–0.59)
-2013–2016		0.37 (0.13–1.00)	0.31 (0.1–1.00)
Seniority of work (years)		1.0 (0.97–1.10)	-
Hours of exposure/ week		1.0 (0.99–1.00)	-

4. Discussion

Our study investigated LA allergy from 2001 to 2016 in Trieste research facilities, showing the progressive reduction of sensitization after the implementation of preventive measures to reduce exposure to animal derivatives. Our population was constituted mainly by young researchers with a mean age below 31 years and few years of exposure (CI95% 2.1 to 5.4). The group was characterized by high percentages of atopic subjects (more than 50% for men involved) with allergic symptoms in an half of them. This percentage is higher than those reported in older studies, where atopic are around 30% [6], though our population can be considered at higher risk to develop LA sensitization and symptoms. On the “already exposed group” sensitization to LA involved 14% of workers, but looking to sensitization trend in years considered, we assisted to a progressive reduction of sensitization with 25.6% in 2001–2004 to 8.2% in 2013–2016. Ferraz et al. [16] in 2013 reported a prevalence of 16.3% of LA sensitization in Brazil, Schmid et al. [17] in 2007 reported a sensitization rate of 12.7 and 16.3% in persons occupationally exposed to mouse and rats, respectively. Previous studies reported higher percentages until 44% of LA allergy in exposed workers [1,18,19].

Factors involved in LA sensitization were atopy (OR 7.1; 95%CI 2.6–19.3) as well as the presence of common allergic symptoms (OR 2.3; 95%CI 1.15–4.7). Atopy is the recognized factor involved in LA allergy [20–24] but also non-atopic subjects can develop LA allergy, in general after longer exposure, as suggested by Kruize et al. [20] in

1997. Time of exposure before the sensitization is in general before 3 years, while after this period risk decreased [20]. In our study the follow-up was completed for an average value of 3.84 ± 1.5 years and incidence cases developed sensitization after 2–8 years of exposure (average 4.4 ± 2.2 years). Nevertheless our population underwent medical examination every year, so early onset of sensitization and symptoms could be lost, despite the accurate evaluation of anamnesis. In a study performed in Sweden [24], the authors found the 19% of sensitization in 2 years of follow-up, with periodical examination every 6 months, suggesting an early sensitization period. Moreover our study showed that the onset of sensitization can occur also after more years of exposure.

The role of exposure, in term of seniority of work in years, or intensity of exposure, in term of hours of exposure to animals per week, did not result a significant related factor for LA allergy. Calendar period was very important, with a significant reduction of LA allergy from 2001 to 2004 to 2009–2012 (OR 0.20; 95%CI 0.07–0.53) and to 2013–2016 (OR 0.37; 95%CI 0.13–1.00) as a positive result of preventive measures adopted, to prevent the incidence of LA allergy. The role of intensity of exposure is debated in literature: Kruize et al. [20] in 1997 reported an increased risk for people exposed between 2 and 15 h per week, compared to those exposed to less than 2 h per week, but higher exposure did not cause an increased risk. The authors explained that as health workers effect; however, they did not find a dose relationship effect between exposure, and onset of LA sensitization. Elliot et al. [6] in 2005 reported a significant increase of LA allergic symptoms for exposures higher than 25 h per week but the dose-response effect was confirmed only for non-atopic workers. Fisher et al. [23] in 1998 did not find any effect of intensity of exposure and only personal history of allergy was significant for the development of LA allergy during the study. More recently Nieuwenhuijsen et al. [7] in 2008, demonstrated a significant increase of any symptoms with the intensity of exposure to rat urinary aeroallergens measured in $\mu\text{g}/\text{m}^3$, while non relationship was found using weekly duration of exposure to rats. Simonetti et al. [8] in 2017 found an increased risk to develop asthma for workers exposed from 3.1 to 5 years compared to those exposed less than 1 year, but they failed to find an increased risk for higher exposure. No other symptoms were significantly related to years of exposures.

The main finding of our study is that the improvement of work conditions and the use of general and personal preventive measures caused a reduction of LA allergy prevalence, however, despite the effort done, 7 incidence cases were reported during follow-up. The positive thing is that in the last 2 years we have only 2 workers sensitized, but without symptoms, while in the past all 5 subjects were positive to LA and reported symptoms work related. Incidence can be considered low, comparing our

Table 5

Results of follow-up investigation (3.84 ± 1.5 years).

	N. 75 subjects	
	1 st examination	final examination
Female N. (%)	50 (66.7)	50 (66.7)
Mean age years ± SD	28.8 ± 7.1	32.9 ± 6.1
Hours per month exposed to LA hours ± SD (CI 95%)	31.1 ± 46.7 (20.1–42.4)	35.2 ± 59.8 (20.7–49.6)
Years exposed to LA years ± SD (CI 95%)	2.9 ± 4.8 (1.7–4.00)	6.1 ± 5.7 (4.6–7.7)
Smokers N. (%)	12 (16)	12 (16)
Students N. (%)	20 (26.7)	7 (9.33)
PhD/Post doc N. (%)	36 (48)	44 (58.7)
Researcher/Professors/Technicians	19 (25.3)	24 (32)
Latex gloves use N. (%)	8 (11.1)	7 (10.2)
Pet owners N. (%)	22 (29.3)	19 (27.1)
Familiar atopy N. (%)	36 (48)	35 (46.7)
Common allergic symptoms N. (%)	23 (30.7)	30 (40)
Oculorhinitis N. (%)	25 (33.3)	26 (34.7)
Asthma N. (%)	12 (16.0)	10 (13.3)
Cough N. (%)	16 (21.3)	16 (21.3)
Urticaria N. (%)	4 (5.3)	4 (5.3)
Dermatitis N. (%)	4 (5.3)	6 (8.0)
Atopy by prick test N. (%)	38 (50.6)	40 (53.3)
Dog N. (%)	17 (22.7)	15 (20.0)
Cat N. (%)	11 (14.7)	12 (16.0)
LA skin prick test + N. (%)	7 (9.3)	11 (14.7)
Mouse N. (%)	4 (5.3)	6 (8.0)
Rat N. (%)	2 (2.7)	6 (8.0)
Guinea pig N. (%)	2 (2.8)	2 (2.8)
Rabbit N. (%)	4 (5.3)	4 (5.3)
Hamster N. (%)	1 (1.3)	3 (4.0)
Latex N. (%)	1 (1.4)	3 (4.0)

Table 6

Factors involved in sensitization to LA during the follow-up evaluated using GEE (generalized equations estimations). In bold significant results.

Factors	OR (CI95%) 1st MODEL	OR (CI95%) 2nd MODEL
Female sex	1.05 (0.32–3.41)	0.79 (0.24–2.57)
Age	1.00 (0.93–1.09)	0.95 (0.83–1.1)
Common allergic symptoms	10.9 (2.54–46.9)	–
Atopy	–	8.1 (1.7–37.4)
Calendar period:		
-2001–2004	1	
-2005–2008	0.23 (0.01–2.86)	
-2009–2012	0.99 (0.01–1.36)	
-2013–2016	0.03 (0.001–0.54)	
Seniority of work (years)		0.99 (0.98–1.01)
Hours of exposure/week		1.12 (0.94–4.4)

results with previous studies, but we are far away to quote “0” sensitization, that must be considered the objective for the future.

Preventive measures must permit to everyone to work with LA, because the onset of sensitization or of symptoms determine the need for subjects to avoid LA exposure and sometimes to leave research

activity. Following good laboratory practices, we suggest that sensitized workers without symptoms can work with LA wearing a protective mask (FFP3) under an extractor hood. Sensitized workers with mild symptoms, such as rhinitis, can work with LA wearing a protective mask, only if this protection is effective to avoid the onset of symptoms, otherwise they must stop LA exposure. Sensitized with important rhinitis, cough or asthma are forbidden to work with animals.

Our study has some strength and some limitations: the strength is the long follow-up that permits to see the effect of improvement of work condition in term of reduction of sensitization; and the number of subjects studied, that represent the 98% of exposed subjects, because medical examinations were compulsory for all exposed workers. The limitations of our study are the periodical medical examination that was done every year, and that the follow-up was possible only for a small group of workers because the majority of them left the study, as they finished the experimental period during the doctorate. Another limitation is the lack of data on exposure; that was evaluated in term of hours and years of exposure but without air monitoring. Despite that, our study demonstrated a progressive reduction of sensitization to LA following good laboratory practices and preventive measures, but confirmed the need to do a periodical medical surveillance screening with skin prick test for workers exposed to LA.

Table 7

Characteristics of subjects sensitized to LA during the follow-up.

First examination	Final examination
6 subjects sensitized to LA	
- 4 with symptoms at work and outside of work, all atopics (3 already exposed; 2 with rhinitis, 1 with asthma and rhinitis, 1 with asthma and cough)	→ - 4 with same symptoms at work and outside of work
- 2 without symptoms	→ - Negative to the final Skin Prick Test, no symptoms
69 subjects not sensitized	→ - 7 incidence cases - 4 with symptoms - 3 asymptomatics

Conflicts of interest

The authors declare that they have no conflict of interests.

Appendix A. Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.rmed.2018.02.002>.

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