

Guillaume Hersen<sup>1</sup>  
Stéphane Moularat<sup>1</sup>  
Enric Robine<sup>1</sup>  
Evelyne Géhin<sup>2</sup>  
Sandrine Corbet<sup>3</sup>  
Astrid Vabret<sup>3</sup>  
François Freymuth<sup>3</sup>

<sup>1</sup>Centre Scientifique et Technique du Bâtiment (CSTB), Laboratoire de Microbiologie des Environnements Intérieurs, Marne-la-Vallée, France.

<sup>2</sup>Université Paris XII, Centre d'étude et de Recherche en Thermique environnement et système, Créteil Cedex, France.

<sup>3</sup>CHU Clémenceau, Laboratoire de Virologie Humaine et moléculaire, Caen Cedex, France.

## Research Article

# Impact of Health on Particle Size of Exhaled Respiratory Aerosols: Case-control Study

Individuals with viral infection could possibly emit an infectious aerosol. The distinction between exhaled breaths of infected and healthy individuals should facilitate an understanding of the airborne transmission of infections. In this context, the present study is aimed at distinguishing healthy individuals from symptomatic ones by the study of their exhaled breath. A setup composed of a modified hood connected to an electrical low pressure impactor, which allows for the study of a wide range of particle sizes (from 7 nm to 10  $\mu\text{m}$ ), has been developed in order to collect exhaled breaths. This setup has been used with seventy eight volunteers. The results obtained using Principal Component Analysis (PCA) showed that exhaled breaths of individuals without symptoms have statistical similarities and are different from those of individuals with symptoms. This separation was made by the greater proportional emission by individuals with symptoms of particles collected on stages 3 ( $D_{50} = 0.09 \mu\text{m}$ ), 6 ( $D_{50} = 0.38 \mu\text{m}$ ), 8 ( $D_{50} = 0.95 \mu\text{m}$ ), 10 ( $D_{50} = 2.40 \mu\text{m}$ ), and 12 ( $D_{50} = 4.02 \mu\text{m}$ ) of the impactor. There was not a specific size distribution obtained for the individuals with symptoms. As a consequence, further research on the exhaled breath should be undertaken with symptomatic volunteers and would require the analysis of this wide range of particle sizes.

**Keywords:** Bioaerosol; Exhaled breath; Particle size; Principle Component Analysis; Virus

*Received:* December 22, 2007; *revised:* February 18, 2008; *accepted:* March 13, 2008

**DOI:** 10.1002/clen.200700189

## 1 Introduction

Respiratory viruses represent a major public health concern. Outbreaks of bronchiolitis, influenza or cold have a major impact on the smooth running of society, e.g., the annual outbreak of bronchiolitis affects more than 460,000 infants in France [1]. Airborne transmission of respiratory viruses has been well established for humans [2–5] and for animals [6–12]. Therefore, it has been shown that infected individuals can emit particles in the air while talking, sneezing or coughing. These droplets may carry microorganisms and are likely to contaminate other people. The size of these droplets determines how long they will stay in the environment before settling and also the pulmonary site of deposition [13–16].

Until recently, only a few studies have explored the size and number of particles emitted during coughing, sneezing or talking. They present various results with the diameters of the droplets ranging between 0.3 and 2000  $\mu\text{m}$ . The first experiments were performed in the early 1940's using high-speed photography [17]. Duguid [18] studied these particles by microscopic measurement of stain marks found on slides exposed directly to air exhaled from the mouth. The study reported that the diameters of the droplets ranged between 1

and 2000  $\mu\text{m}$ . It was also stated that 95% of these particles were less than 100  $\mu\text{m}$  and that most droplets were between 4 and 8  $\mu\text{m}$  in diameter. The number of test subjects and their health status were not described.

Loudon and Roberts [19] used a chamber with bond paper placed inside to study droplet size. Three subjects with a dye in their mouths coughed into this chamber. After 30 min, the air was withdrawn from inside the chamber through a filter. Following this, stain marks on the bond paper and the filter were measured using microscopy. In this work, the average number of particles emitted per cough was 470. Particles with a diameter of less than 10  $\mu\text{m}$  represented 53.6 % of the sample. The health status of the test subjects was not described.

Gerone et al. [20] used a 127 L stainless steel chamber shaped as a truncated cone to minimize the impaction of particles on its sides. It was then associated with different systems, e.g., impinger, impactors and a particle-size analyzer, to collect coughs and sneezes. In the study, the volunteers were experimentally infected with coxsackievirus A-21. The authors reported that most of the particles were less than 1  $\mu\text{m}$ .

Papinen and Rosenthal [21] used an optical particle counter (OPC) connected to a funnel into which five healthy subjects coughed. Size spectra were reported to be weighted towards the smallest particles detectable by the OPC (the OPC was reported to have a lower diameter limit of 0.3  $\mu\text{m}$ ). Approximately 85% of the particles detected had diameters of less than 1  $\mu\text{m}$  and the total droplet concentrations ranged from 1 to 218 per L. Finally, Fennelly

**Correspondence:** E. Robine, Centre Scientifique et Technique du Bâtiment (CSTB), Laboratoire de Microbiologie des Environnements Intérieurs, 84, av. Jean Jaurès, Champs-sur-Marne, 77447 Marne-la-Vallée, France.

**E-mail:** enric.robine@cstb.fr

et al. [22], using a chamber containing microbial air samplers, showed that most particles produced by individuals with pulmonary tuberculosis were in the respirable size range.

Since methods and test subjects are different from one study to another, it is difficult to compare these results. Consequently, detailed information is not available. In particular, it is not possible at present to determine the impact of volunteer health on the particle size of exhaled respiratory aerosols. In this context, the present study is aimed at distinguishing healthy individuals from symptomatic ones by the study of their exhaled breath.

The experimental approach consisted of firstly developing a system that allows measurement of fine particles exhaled from a greater numbers of volunteers (78 individuals), with and without, symptoms. Following this, the size differences between aerosols emitted by symptomatic volunteers and controls were determined. Finally, confounding factors, i.e., factors that could distinguish between the two groups of volunteers without one noticing were researched.

## 2 Materials and Methods

### 2.1 Human Volunteers

Seventy eight human volunteers, ranging in age from 6 to 66 years, participated in this study. This panel was composed of 43 patients selected by a general practitioner, called “symptomatics”, with objective symptoms, i.e., cough, cold, etc. and 35 healthy persons without symptoms called “controls”. Among the population, 54% of the symptomatic volunteers and 50% of the controls were women.

### 2.2 Administered Questionnaire

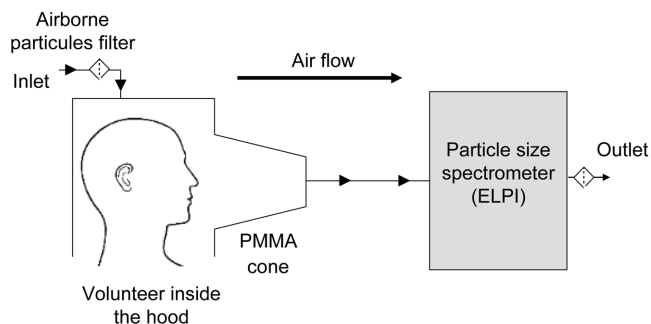
Information was collected using a face to face interview with volunteers. Eight questions were asked: three questions concerned volunteers themselves (sex, age, and smoking habits) and five questions described the symptoms (headache, fever, running nose, loose cough and dry cough).

### 2.3 Biological Analyzes

Biological analyzes were performed for each volunteer on a nasal swab using the Polymerase Chain Reaction (PCR) method. The viruses searched were influenza viruses A, B, and C, Respiratory Syncytial Virus (RSV), Human Metapneumovirus (HMPV), Rhinovirus, Enterovirus and Coronavirus [23].

### 2.4 Description of the Setup Designed for Aerosol Collection

A specific apparatus was designed in order to collect exhaled breaths [24]. This experimental system is outlined in Fig. 1 and is comprised of a modified cylindrical polyurethane hood and hose (Protector Tornado T-9 hood, Scott Health and safety, Lancashire, England) ventilated with powered air (Protector Tornado T-Power, Scott Health and Safety, Lancashire, England) connected to a Poly-Methyl MethAcrylate (PMMA) cone shaped part (BFP-Cindar, Champigny-sur-Marne, France). The initial flow rate inside the hood of 140 L/min was decreased to 40 L/min in order to limit the particle loss. In order to prevent the particle content in the room obscuring



**Figure 1.** Setup for the collection of the exhaled breath of volunteers coughing inside the ventilated hood.

the particles produced by coughing, the air in the hood was filtered with a P3 particulate filter (PSL filter PF251/2, Scott Health and Safety, Lancashire, England). This modified ventilated hood was linked to an Electrical Low Pressure Impactor (ELPI, Dekati, Tampere, Finland). The ELPI is a real-time particle size spectrometer. It measures airborne particles in the concentration size range of  $7 \cdot 10^{-3}$  to  $10 \mu\text{m}$  (see Tab. 1). Its nominal airflow is 10 L/min. A membrane filter (Durapore membrane filter, Millipore, MA, USA) was placed on each stage of the ELPI in order to recover collected particles. No grease was added.

### 2.5 Particle Loss in the System

In order to determine the particle loss in the system, an aerosol was compared before and after its passage through the ventilated hood. In this way an aerosolization setup, was positioned either directly connected to the ELPI (to represent the aerosol before its passage through the hood) or inside the hood connected to the ELPI. The aerosolized suspension was a ten-fold dilution of a solution that simulates saliva (Artisial, Laboratoire Chemineau). The aerosol was generated using a Collision nebulizer at 20 psi (BGI, MA, USA). Then it was mixed with dry filtered air (10 L/min) in a homogenization sphere (Belleville, France). Each aerosolization lasted for 2 min.

### 2.6 Test Protocol

Experiments were the same for each volunteer. After answering the questionnaire, individuals were placed inside the ventilated hood, which was connected to the ELPI. Volunteers were asked not to cough during the first 10 s of the experiment in order to obtain the background noise. They were then invited to cough as much as they could and for as long as possible. Experiments were stopped when volunteers requested it. The ventilated hood was cleaned using ethanol following each experiment.

### 2.7 Statistical Analysis

#### 2.7.1 Principal Component Analysis (PCA)

A size distribution corresponding to the particles emitted by each volunteer was obtained. The concentrations of these profiles were extracted. The result takes the form of a matrix of 79 lines or observations (78 volunteers) and 11 columns or variables (10 ranges of particle size). The intersection of a line and a column represents the

**Table 1.** ELPI channel descriptions and correction factors assigned to volunteers' emissions ( $D_{50}$ : Cutpoint, Aerodynamic diameter,  $\mu\text{m}$ ).

Stage	Terminal filter	1	2	3	4	5	6	7	8	9	10	11	12
Cutpoint $D_{50}$ ( $\mu\text{m}$ )	0.01	0.03	0.06	0.09	0.16	0.26	0.38	0.62	0.95	1.61	2.40	4.02	9.97
Correction factor	–	1.95	2.73	3.56	1.77	1.50	1.11	1.08	0.95	0.82	0.78	5.92	14.80

particulate concentration obtained for one stage of the ELPI throughout the size distribution for each volunteer.

The dimensions of this data matrix are such that it is impossible to directly detect any similarities in statistical behavior between the volunteers (individuals) or sizes (variables). Principal Component Analysis (PCA) [25] was chosen to analyze these results. This type of analysis was employed in other studies processing the results obtained using data from microorganisms (mould or bacterium) [26, 27]. It enables a statistical representation of all data without emitting any hypothesis. PCA generates an optimum and similar graphical representations of the scatterplot representing the data matrix. Each PCA factor is a linear combination of variables representing the maximum variance in the scatterplot. Factors have an orthogonal relationship, so that they only take independent sources of variance into account. Therefore, two spaces are constructed with the same factors, i. e., a vectorial space for variables and an observation space. Two factors define a plane on which points (observations) or vectors are projected. The proximity of two vectors (variables) indicates a strong linear correlation between these two variables, as does the proximity of two points. Therefore, groups of observations and variables are defined to provide a view of the data structure that is little evident at first sight. SPAD version 3.5 (Décisia) data analysis software was used for these analyzes.

### 2.7.2 Multiple Correspondence Analyzes (MCA)

The answers of the volunteers to the questionnaire were transformed into a  $79 \cdot 9$ -point matrix. In this matrix, lines represent the volunteers and columns represent the questions. The intersection of a line and a column represents the answer to each question. The answers are binary, i. e., yes or no.

A Multiple Correspondence Analysis (MCA) was applied to this matrix in order to identify the association between the selected factors. MCA is an extension of Correspondence Analysis (CA) to the case of more than two variables. It is a method that allows the study of the association between two or more qualitative variables. MCA represents for qualitative variables what Principal Component Analysis does for quantitative variables. One can obtain maps where it is possible to visually observe the distances between the categories of the qualitative variables and between the observations [28].

## 3 Results and Discussion

### 3.1 Experimental Data Corrections

#### 3.1.1 Limit of Detection (LOD)

The limit of detection (LOD) of the system was determined on 30 measures of blank (air filtered from the hood with nobody inside). The LOD is defined by Eq. (1):

$$\text{LOD} = 3 \cdot [\text{Standard deviation of the blank}] \quad (1)$$

In doing so, it was estimated that the LOD was  $140 \text{ particles cm}^{-3}$ . Only emissions with total concentrations greater than this value were considered. Moreover, the background noise, i. e., the concentration measured before the volunteer starts to cough, was removed from the measured concentration during the emission for each stage of the ELPI.

#### 3.1.2 Correction Factors

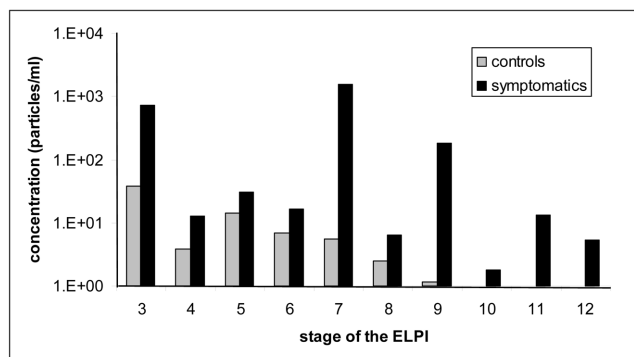
In order to estimate the particle loss in the system, aerosol mimicking saliva was compared before and after its passage in the modified hood. These experiments ( $n = 5$ ) permitted the assignment of a correction factor for each channel of the ELPI to the emissions of each volunteer. Table 1 lists the correction factors obtained for each stage of the ELPI.

### 3.2 Impact of the Symptoms on the Size Distribution of the Particles Emitted by Volunteers

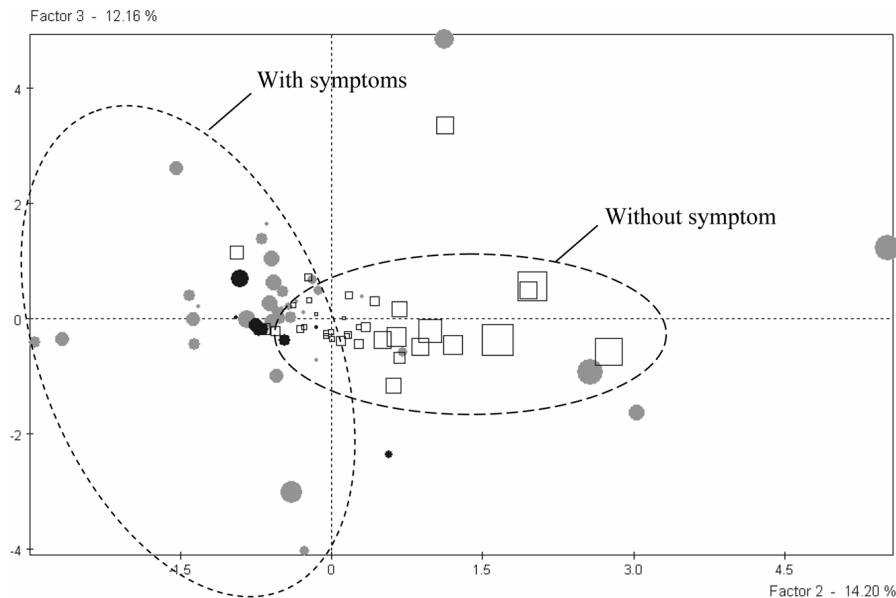
The results of the biological analysis highlighted eight symptomatic volunteers infected by influenza virus and one by corona virus. No virus was found among the controls. Due to the fact that the size of the influenza virus and corona virus are greater than  $80 \text{ nm}$  [1] the first and second channels of the ELPI (see Tab. 1) were not considered afterwards. The averaged size distributions of emissions are presented for each group in Fig. 2.

This representation reveals that symptomatic volunteers emit more particles than controls, especially considering particles of  $0.5 \mu\text{m}$  and micronics particles. These particles, considering their size, could carry some microorganisms such as virus or bacteria. These results are in accordance with the study of Fennelly et al. [22]. However, the important dispersions of concentrations inside each group (210% for each group) make it difficult to draw conclusions just by the global observation of the two groups.

Moreover, analysis of the data collected consists of comparing two series of more than 40 size distributions, where each represents



**Figure 2.** Averaged particulate concentrations (particles/mL) of emissions measured on ELPI stages for each group of volunteers.



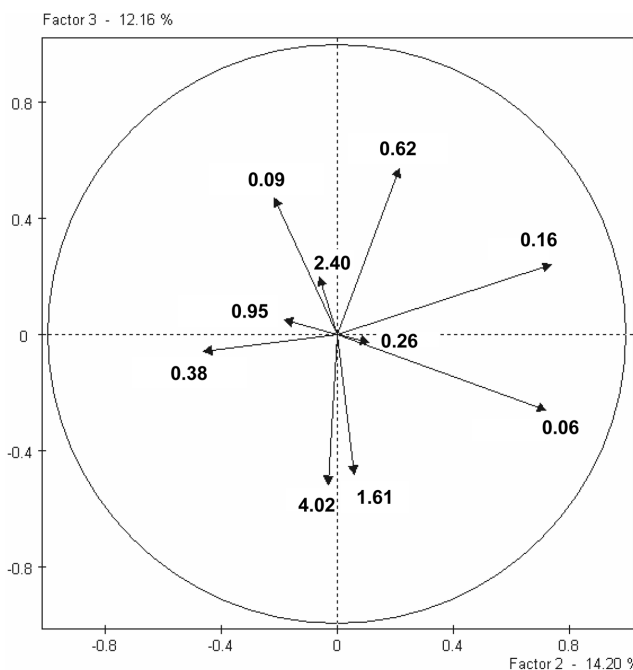
**Figure 3.** Diagrammatic representation of a PCA made on 78 volunteers. □: Individuals without symptom, ○: Individuals with symptoms, and ●: Infected volunteers.

a significant quantity of information. Therefore, PCA, which enables a simple representation of data, was employed in order to study variances in detail without emitting hypothesis, see Fig. 3. Data were standardized before applying the PCA due to their important dispersion. Therefore, the concentration of each particle size was divided by the total concentration for each of the 78 volunteers.

The diagrammatic representation obtained using the SPAD program reveals two groups of individuals, which are outlined by drawing two ellipses. These groups are composed of individuals with and without symptoms, respectively. A close comparison between the two groups reveals that symptomatics are more dispersed than controls. Indeed, symptomatics scatter along the two axes while “controls” only scatter along the first axis. As expected, individuals with viral infections are among the symptomatics. However, the presence of a virus had no impact on the exhaled breath, and therefore, infected people did not form a third group. Nevertheless, it is difficult to conclude on the impact of virus considering the low number of infected volunteers involved. This method facilitated the separation for 80% of individuals.

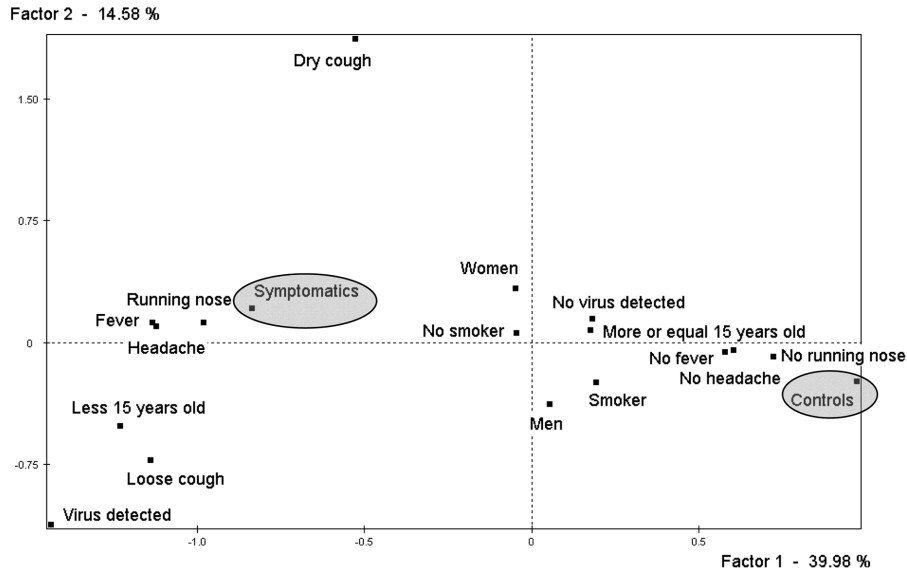
It should be noted that these results were obtained using the size distribution of the particles emitted in the exhaled breaths. As a result, the partition observed above is the consequence of the differences in these size distributions. As can be seen in Fig. 3, it is essentially the second factor that distinguishes the two groups. This factor corresponds to several stages of the impactor (variables). In order to identify these variables, they were projected onto a correlation circle, see Fig. 4. The length of the vectors obtained provides information on their representativeness in the circle defined by the principal plane. Thus, the greater the importance of the vector norm, the more a variable is well represented in the chosen plane.

By comparing the position of the volunteers on the PCA representation, Fig. 3, with the directions of vectors onto the correlation circle, see Fig. 4, it is possible to determine which variables are responsible of the separation. In doing so, it should be noted that five stages of the impactor are involved. Their cutpoints ( $D_{50}$ ) are 0.09, 0.38, 0.95, 2.40, and 4.02  $\mu\text{m}$ , respectively. These results indicate that, in this study, symptomatics emitted more of these particles than controls.



**Figure 4.** Projection of the impactor’s stages that compose the second factor (factor that separates the two groups of volunteers) onto a correlation circle. Each arrow represents an impactor stage and is identified by its cutpoint ( $D_{50}$ ,  $\mu\text{m}$ ).

The fact that symptomatics are more dispersed than controls, Fig. 3, shows that they did not present a single type of exhaled breath. They can differ from the controls because of one or more of the particle sizes determined above. In 1997, Papineni and Rosenthal [21] supposed that, owing to their small size, viruses were susceptible to being among the fine particles, but until now there was no device that could measure fine particles. Using the ELPI, it appears that exhaled breaths contain fine particles that are possibly infectious. In addition, the data obtained with the PCA permitted highlighted



**Figure 5.** Diagrammatic representation of a MCA made by using the administered questionnaire in order to find out potential confounding factors. A confounding factor is revealed by the superposition of points.

the necessity to take into account both the fine particles and also super-micronic particles.

### 3.3 Research of Confounding Factors

In this part of the study, the presence of possible confounding factors, which are other factors that could cause the separation between the two groups without one noticing, was checked. Sixteen factors were compared with symptomatics factor and controls using the MCA method. In the diagrammatic representation, a confounding factor is revealed by the superposition of points. The findings are summarized in Fig. 5. There is no superposition between symptomatics or controls and others factors. Hence, there is no confounding factor concerning the two factors studied here, i.e., symptomatics and controls. In the same way, “sex” and “smoker” characters (near the origin in this graph) are halfway between symptomatics and controls. Thus, they have no effect on the separation observed in the previous graph. Finally, the symptomatics factor is halfway between the “loose cough” factor and the “dry cough” factor. Subsequently, the size distributions of the symptomatics are independent of the type of cough.

## 5 Conclusions

The purpose of this work was to distinguish healthy individuals from symptomatics by the study of their exhaled breath. From this preliminary study, it can be concluded that, on the one hand, exhaled breaths of individuals without symptoms have statistical similarities and are different from those of individuals with symptoms. On the other hand, a specific size distribution is not obtained for the individuals with symptoms. It was shown that a proportionally greater emission by symptomatic individuals of particles collected onto the impactor stages with cutpoint ( $D_{50}$ ) of 0.09, 0.38, 0.95, 2.40, and 4.02  $\mu\text{m}$  make this separation.

To the current authors' knowledge, differences between aerosols produced by symptomatic volunteers and controls have not been studied previously. Moreover, this is the first time that such a large

panel was used (78 volunteers) to study the exhaled breath [18–21]. The knowledge of the exhaled breath of symptomatic individuals should also permit a better understanding of airborne transmission mechanisms, in particular, considering annual outbreaks and pandemic threats.

### Acknowledgements

The authors thank Marie-Noël Grattepanche for the selection of the volunteers with symptoms. Olivier Ramalho and Jean-Paul Lucas provided excellent advice in PCA and MCA.

### References

- [1] J.-M. Huraux, J.-C. Nicolas, H. Agut, H. Peigue-Lafeuille, *Traité de Virologie Médicale*, Estem, Paris 2003, 686.
- [2] R. H. Alford, J. A. Kasel, P. J. Gerone, V. Knight, Human influenza resulting from aerosol inhalation, *Proc. Soc. Exp. Biol. Med.* **1966**, 122, 800.
- [3] T. R. Cate et al., Production of tracheobronchitis in volunteers with rhinovirus in a small-particle aerosol, *Am. J. Epidemiol.* **1965**, 81 (1), 95.
- [4] R. B. Couch et al., Effect of route of inoculation on experimental respiratory viral disease in volunteers and evidence for airborne transmission, *Bacteriol. Rev.* **1966**, 30, 517.
- [5] M. R. Moser et al., An outbreak of influenza aboard a commercial airliner, *Am. J. Epidemiol.* **1979**, 110 (1), 1.
- [6] E. Bourgueil, E. Hutet, R. Cariolet, P. Vannier, Experimental infection of pigs with the porcine respiratory coronavirus (PRCV): measure of viral excretion, *Veterinary Microbiol.* **1992**, 31, 11.
- [7] S. L. Brockmeier, K. M. Lager, Experimental airborne transmission of porcine reproductive and respiratory syndrome virus and *bordetella bronchiseptica*, *Veterinary Microbiol.* **2002**, 89, 267.
- [8] M. A. S. Y. Elazhary et al., Experimental infection of calves with bovine respiratory syncytial virus (Quebec strain), *Can. J. Comp. Med.* **1980**, 44, 390.
- [9] C. S. Kristensen et al., Experimental airborne transmission of PRRS virus, *Veterinary Microbiol.* **2004**, 99, 197.
- [10] M. H. Mars, C. J. M. Bruschke, J. T. Van Oirschot, Airborne transmission of BHV1, BRSV, and BVDV among cattle is possible under experimental conditions, *Veterinary Microbiol.* **1999**, 66, 197.

- [11] M. H. Mars et al., Airborne transmission of bovine herpes virus 1 infections in calves under field conditions, *Veterinary Microbiol.* **2000**, 76, 1.
- [12] P. Otto et al., A model for respiratory syncytial virus (RSV) infection based on experimental aerosol exposure with bovine RSV in calves, *Comp. Immun. Microbiol. Infect. Dis.* **1996**, 19 (2), 85.
- [13] V. Knight, Airborne transmission and pulmonary deposition of respiratory viruses, *VI<sup>th</sup> Int. Symp. on Aerobiology*, **1973**, pp. 175–182.
- [14] M. G. Ménache, F. J. Miller, O. G. Raabe, Particle inhalability curves for humans and small laboratory animals, *Ann. Occup. Hyg.* **1995**, 39 (3), 317.
- [15] P. E. Morrow, Physics of airborne particles and their deposition in the lung, *Ann. N. Y. Acad. Sci.* **1980**, 353, 71.
- [16] A. Renoux, D. Boulaud, *Les Aérosols; Physique et Métrologie*, Lavoisier Tec et Doc, **1998**, 301.
- [17] C. E. Turner, M. W. Jennison, H. E. Edgerton, Public health applications of high-speed photography, *Am. J. Public Health Nations Health* **1941**, 31, 319.
- [18] J. P. Duguid, The size and duration of air carriage of respiratory droplets and droplet nuclei, *J. Hyg.* **1946**, 44, 471.
- [19] R. G. Loudon, R. M. Roberts, Droplet expulsion from the respiratory tract, *Am. Rev. Respir. Dis.* **1967**, 95, 435.
- [20] P. J. Gerone et al., Assessment of experimental and natural viral aerosols, *Bacteriol. Rev.* **1966**, 30, 576.
- [21] R. S. Papineni, F. S. Rosenthal, The size distribution of droplets in the exhaled breath of healthy human subjects, *J. Aerosol Med.* **1997**, 10 (2), 105.
- [22] K. P. Fennelly et al., Cough-generated aerosols of *Mycobacterium tuberculosis*: a new method to study infectiousness, *Am. J. Respir. Crit. Care Med.* **2004**, 169, 604.
- [23] S. Bello-Pujol et al., Development of three multiplex RT-PCR assays for the detection of 12 respiratory RNA viruses, *J. Virol. Methods* **2005**, 126 (1–2), 53.
- [24] G. Hersen et al., Etude de l'exposition aux aérosols viraux dans les environnements intérieurs, *Congrès Français sur les Aérosols*, Paris **2006**.
- [25] L. Lebart, A. Morineau, M. Piron, *Statistique Exploratoire Multidimensionnelle*, 2nd ed., Dunod, Paris **1997**, p. 436.
- [26] S. Moularat, Etude de la contamination fongique des environnements intérieurs par la détermination et la mesure de traceurs chimiques spécifiques: application à l'hygiène de l'habitat, *Ph.D. Thesis*, Université de Marne-la-Vallée **2005**.
- [27] S. Park et al., Principal component analysis and discriminant analysis (PCA-DA) for discriminating profiles of terminal restriction fragment length polymorphism (T-RFLP) in soil bacterial communities, *Soil Biol. Biochem.* **2006**, 38, 2344.
- [28] M. Greenacre, J. Blasius, *Multiple Correspondance Analysis and Related Methods*, Chapman Hall, Boca Raton **2006**, p. 608.