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Epidemiology of Viral Infections and Evaluation of the Potential Benefit of OM-85 BV on the Virologic Status of Children Attending Day-Care Centers

Abstract

Viral investigations were performed during 4 winter seasons (88/89, 89/90, 92/93, 93/94) in children attending day-care centers (DCCs) in the Rhône Département in eastern France. Over the total observation period of 4 winter seasons, 780 children were screened with a nasal swab for the presence of viruses. Of those, 230 (29.5%) had a positive viral culture. The viruses identified were respiratory syncytial virus (RSV), influenza A and B virus, parainfluenza virus, coronavirus, rhinovirus, adenovirus and enterovirus. During that time, 83 epidemic events in 47 DCC were recorded. A particular virus was judged to be causally related to an epidemic if the identical virus was isolated in ≥ 3 children during the same outbreak of respiratory diseases. Thus, in 51 cases (61.4%) of all epidemics, the following viruses were responsible for an epidemic: RSV (n = 23), coronavirus (n = 10) (only during the season of 1993–1994), influenza A virus (n = 6), rhinovirus (n = 4), enterovirus (n = 4), adenovirus (n = 3) and parainfluenza virus (n = 1). Except for the somewhat surprising accumulation of coronavirus epidemics during the winter of 1993–1994, there were only minor seasonal variations from one year to another. As expected, RSV accounted for about one third of all respiratory tract infections in children attending DCCs and was therefore the most important single causative agent. These results are compared with data from children who did not attend a DCC and were cared for in a private practice. During the winter of 1989–1990, the viral epidemiological survey was performed at the same time and in parallel to a double-blind, placebo-controlled clinical study investigating the efficacy of OM-85 BV, an immunoactive bacterial extract. This study, enrolling 423 children attending DCCs demonstrated a protective effect of OM-85 BV in significantly reducing the risk of recurrent infections of the upper respiratory tract during the treatment period with the compound. 34% of all participating children (75 in the verum group, 70 in the placebo group) were enrolled in an additional virological study. In these patients, RSV was isolated 10 times in the placebo group, but only 5 times in the treated group ($p < 0.05$) and influenza A virus was present in 4 children in the placebo group, but only in 1 infant in the verum group giving a total of 14 positive virologic results in the placebo group versus 6 in the verum group ($p < 0.05$). Despite the small numbers of children investigated for their virologic status during respiratory infectious outbreaks, there was a statistically significant difference in the prevalence of virus carriers in favor of the children treated with OM-85 BV. These results corroborate the clinical findings.

Key Words
OM-85 BV
Respiratory viruses
Day-care centers
Children

Introduction

An increasing number of children are attending the public care systems enabling mothers to pursue or resume a professional activity because of economical, social or personal reasons. France disposes of a dense network of day care centers (DCCs) which are directed and operated by specially trained personnel.

It is well established that DCCs are associated with a higher risk for the attending children to contract different

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infectious diseases than in other social environments and that this risk depends on different factors. The risk of developing a first episode of common cold with fever, otitis or wheezy bronchitis in the early months following admission to a DCC is significantly higher in these infants than in children who stay at home [1]. Other recent surveys implemented in Pennsylvania [2, 3] and Georgia [4, 5] also demonstrated that the risk of respiratory illnesses associated with DCC attendance was significantly higher for the youngest children. The main risk factor is age, this being related to the disappearance of the protecting maternal antibodies within 3–4 months after birth rendering the infants susceptible to new infectious agents and leaving them with a still immature immune system.

Other risk factors include seasonality – winter is associated with a higher incidence of respiratory infections – and the type of day-care setting. In a large epidemiological study entitled 'Santé-Enfant-Crêche' ('Health-Child-Day-Care'), 1,242 children aged 3 months to 3 years were enrolled between October 1988 and June 1989. During that period, out of a total of 3,639 infectious episodes, 2,916 infections of the upper respiratory tract (URT) were recorded, representing 80.1% of all episodes. The mean number of URT diseases per child varied depending on the type of DDC: 2.78 infectious episodes per child for the larger DCCs (>40 children), 3.47 for the small DCCs (20–40 children) and 2.46 for family day care. These figures contrast with the incidence of infectious episodes for children cared for at home: 0.63 [6].

Viruses are the most frequent etiologic agents for respiratory tract infections in the first year of life, of which the respiratory syncytial virus (RSV) is well known to be the major cause [7–9]. An abundant literature establishes its responsibility in bronchiolitis that may lead to hospitalization in severe cases and occasionally requires specialized intensive care. Today, death is rare but remains a threat [5].

The results of the viral epidemiological investigations over 4 winter seasons in DCCs in the Rhône Département are presented and discussed together with the results of a preliminary study on the potential benefit of OM-85 BV, an immunoactive bacterial extract, on the viral infections in children attending DCCs.

Material and Methods

For practical and logistical reasons, the laboratory diagnosis of viral infection was restricted to so-called 'epidemic events'. These were defined as ≥25% of all the children attending a DCC, or at least 4 children in the same DCC falling sick and presenting the same clinical symptoms within a 3-day period. As during the winter of 1992–1993 only 12 epidemic events were recorded, the criteria for the next season were somewhat modified: ≥20% of the children attending a DCC or 3 children showing the same symptoms, over a period of 3 days. In this case, all children included in the study were examined and a nasal swab was taken (patients and contacts) and a trained nurse was visiting the sick children staying at home, to collect the nasal swabs (Virocult, Medical Wire and Equipment Potley-Corsham, Wilks., UK).

The samples reached the laboratory within a few hours. From an aliquot of the nasal sample, cells were centrifuged for 5 min at 15,000 *g*, diluted in a small amount of PBS, distributed in a microwell plate, at the rate of about 10,000 cells/well × 3 wells and fixed with cold acetone (fig. 1). In a rapid direct diagnostic test detecting antigens by immunofluorescence using monoclonal antibodies, we scored the percentage of fluorescent ciliated cells from 1+ to 4+. For RSV, we used an FITC-labelled monoclonal antibody (RSV direct IF, Biomérieux), whereas for adenovirus and coronavirus, we made an indirect immunofluorescence test using respectively the clone Bioll, Biosoft and a monoclonal antibody (anti-feline infectious peritonitis coronavirus) kindly supplied by Dr. Chappuis, Rhône-Mérieux, and an FITC-conjugate goat anti-mouse IgG + IgM (Tago).

In another aliquot of the nasal swab, we searched for antigens with an Elisa immunocapture test [10, 11]. For influenza A, we used the monoclonal antibody NPA 153 kindly supplied by A. Douglas (W.I.C., London, UK) for influenza B, a monoclonal antibody (8F1OE10) produced in house, for the parainfluenza viruses 1 + 2 + 3, a supernatant of hybridoma cell culture (GF8D) produced in house, and the respective polyclonal antisera prepared in rabbits immunized with purified concentrated viruses mixed with complete Freund's adjuvant. The optical density was recorded and allowed a measurement of the amount of viral antigen present in the sample.

A third aliquot of sample was treated with a mixture of antibiotics and inoculated in cell cultures by low-speed centrifugation at 700 *g* for 30 min. Inoculated cell cultures MDCK and LLCMK2 in the presence of trypsine, Hep2, MRCS, vero, were incubated for 4 days at 34°C, then viral antigens were searched for in the medium by Elisa immunocapture tests (parainfluenza 1, 2, 3, influenza A, B, adenovirus). During the 12 days of incubation, viral growth was looked for by CPE for adenovirus, rhinovirus, coronavirus, enteroviruses and others, by hemadsorption for influenza, parainfluenza and measles viruses. If positive, the cell mixture and the medium were subcultured in order to identify the viruses by classical tests (hemagglutinin inhibition, neutralization, pH lability) [12].

Results of the Epidemiological Surveys

Over the total observation period of 4 winter seasons, 1,425 children in 107 DCCs were followed. During that time, 83 epidemic events in 47 DCCs were recorded and 780 children (54.8%) were screened with a nasal swab for the presence of viruses (table 1). As explained above, the definition 'epidemic event' was changed for the last season of 1993–1994 leading to a significant increase of all recorded data.

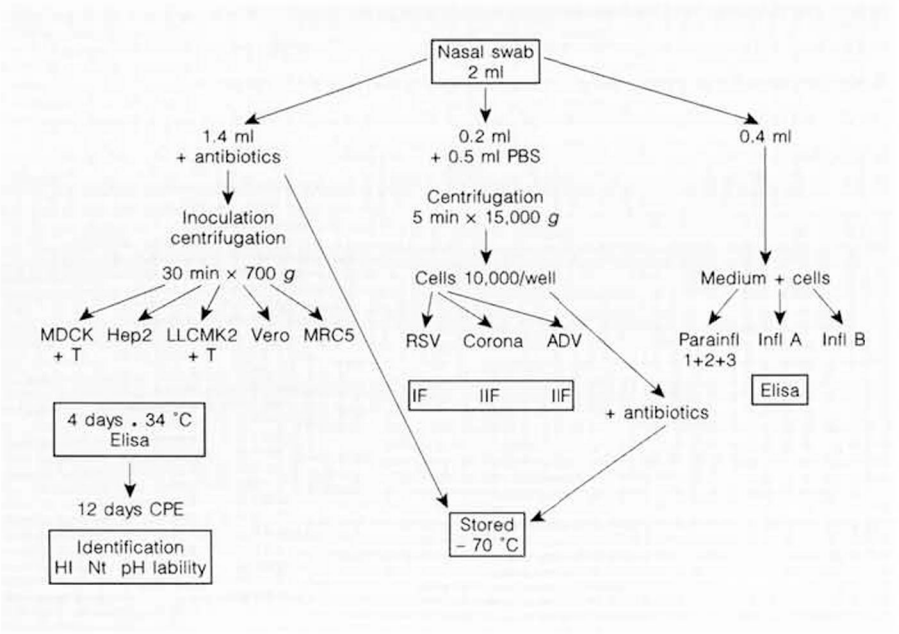


Fig. 1. Viral diagnostic procedure, for details see text.

A positive viral culture was found for 230 children (29.5%). The viruses identified were respiratory syncytial virus (RSV), influenza A and B virus, parainfluenza virus, coronavirus, rhinovirus, adenovirus and enterovirus. The detailed breakdown is given in table 2. The seasonal distribution of the individually isolated viruses is graphically illustrated in figure 2. It confirms the peak, in winter, of RSV and influenza A viruses and shows that coronaviruses were also spreading mainly in winter. Rhinoviruses were predominant in November and February as were adenoviruses. A single case of influenza B was detected in mid-February 1993 and echoviruses were found to provoke outbreaks also in winter and not only in spring [13].

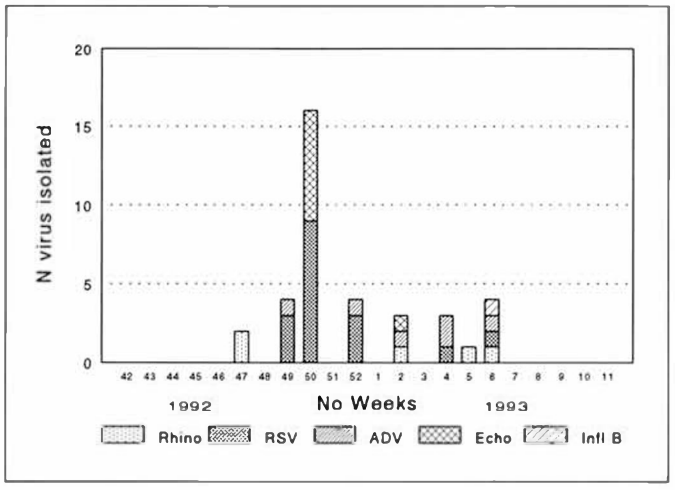
The seasonal distribution of the epidemics is shown in fig. 3. It has to be mentioned that most of the DCCs were closed over Christmas/New Year, explaining the very low incidence of events registered during that period. A particular virus was judged to be causally related to an epidemic if the same virus was isolated in ≥ 3 children during the same outbreak of respiratory diseases (table 2). In 51 of 83 epidemics during the 4-year period, the following viruses were responsible for an epidemic: RSV (n = 23), coronavirus (n = 10) (only during the season 1993–1994), influenza A virus (n = 6), rhinovirus (n = 4), enterovirus (n = 4), adenovirus (n = 3) and parainfluenza virus (n = 1) (fig. 4).

As expected, RSV accounted for about one third of all respiratory tract infections in children attending DCCs and is therefore the most important single causative agent. The other viruses are submitted to more or less important variations from one year to another and coronaviruses were largely predominant during the last winter. In 1993–1994, influenza A H3N2 provoked 4 outbreaks and was isolated sporadically during 10 other epidemic episodes, whereas in 1989–1990 influenza A H3N2 provoked only 2 outbreaks in DCCs. Interestingly, influenza B clinically affected only one single child in the winter of 1992–1993.

Table 1. Comparison of the epidemics of respiratory infections over 4 winter seasons in DCCs in the Rhône Département

Year	DCCs	Children	Epidemics per DCC	Children sampled	Virus isolation
88–89	27	420	12/11	103	42 (40.8)
89–90	27	423	17/11	206	53 (25.7)
92–93	33	339	12/10	91	37 (40.7)
93–94	20	243	42/15	380	98 (25.8)
Total	107	1,425	83/47	780	230 (29.5)

Children sampled = children presenting with clinical symptoms of respiratory infection; Virus isolation = either in nasal swab (all viruses) or in feces (only adenovirus and echovirus). Figures in parentheses are percentages.



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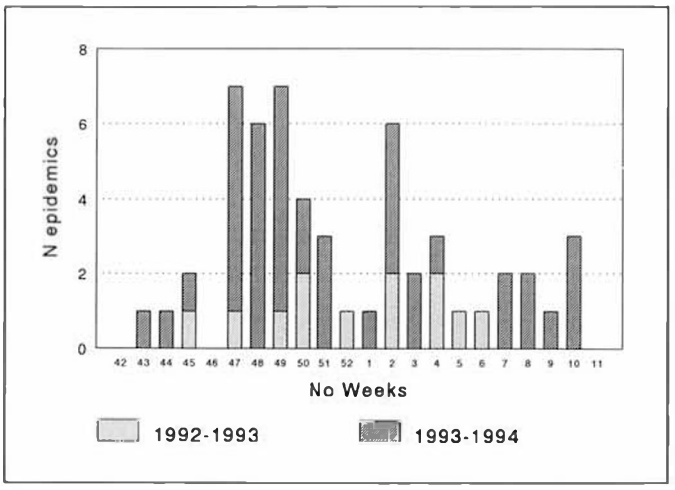

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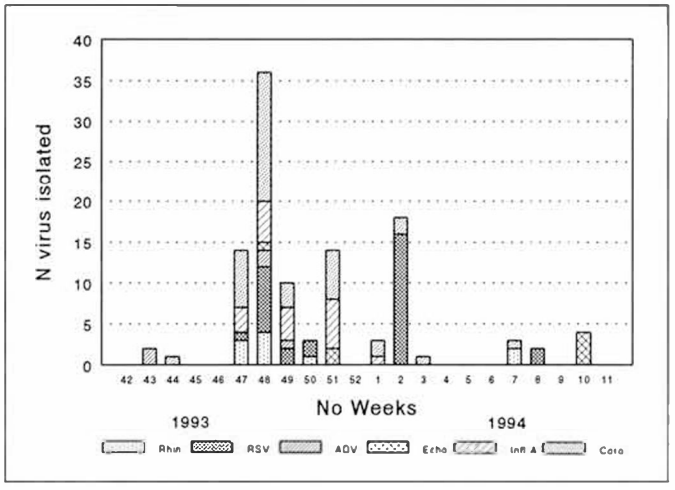

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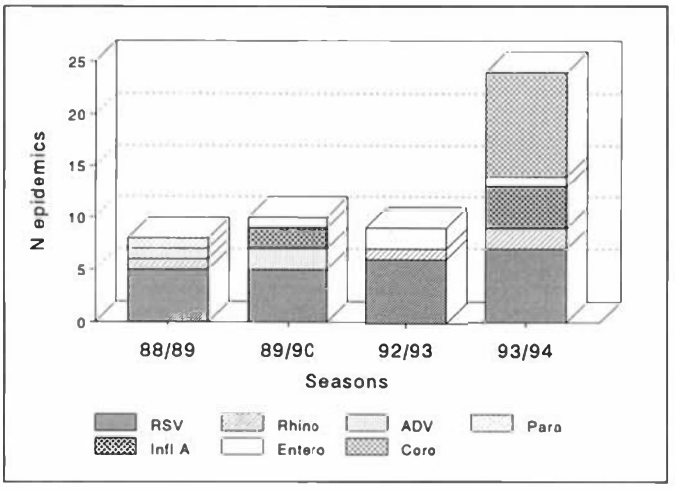

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Fig. 2. Seasonal distribution of all the individually isolated viruses over the two winters, 1992–1993 (**a**) and 1993–1994 (**b**). Rhino = rhinovirus; RSV = respiratory syncytial virus; ADV = adenovirus; Echo = echovirus; Infl A = influenza A virus; Infl B = influenza B virus; Coro = coronavirus.

Fig. 3. Cumulative seasonal distribution of the epidemic episodes over the two winters, 1992–1993 and 1993–1994.

Fig. 4. Viruses identified as probable causative agent of a particular epidemic; isolation of ≥3 times the same virus for a given epidemic (for abbreviations see fig. 2).

Table 2. Viruses responsible for the epidemics of respiratory infections in DCCs

Year	Epi	RSV	Influenza		Para-I	Coro	Rhino	ADV	Entero	Undet
			A	B						
88–89	12	5	0	0	1	ND	1	1	0	4
89–90	17	5	2	0	0	ND	0	2	1	7
92–93	12	6	0	0	0	0	1	0	2	3
93–94	42	7	4	0	0	10	2	0	1	18
Total	83	23	6	0	1	10	4	3	4	32

In order to attribute a particular virus to an outbreak, the same virus had to be isolated ≥3 times. Epi = Number ofepidemics; Para-I = parainfluenza virus; Coro = coronavirus; Rhino = rhinovirus; ADV = adenovirus; Entero = enterovirus; Undet = undetermined.

Table 3. Mixed viral infections during the seasons of 1992–1993 and 1993–1994

Year	Association	n
1992–1993	RSV-Entero	1
	RSV-Rhino	1
1993–1994	RSV-Rhino	2
	RSV-Corona	5
	RSV-Corona-Rhino	1
	Corona-Adeno	1
	Corona-Rhino	1
	Corona-Influenza A	4

Abbreviations, as in table 2.

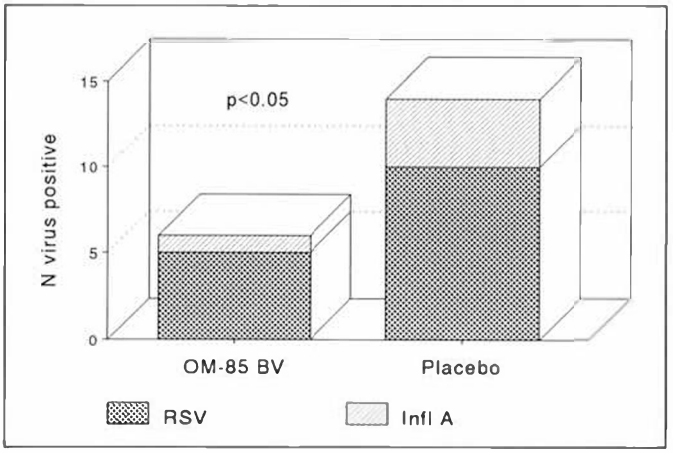

Fig. 5. Number (N) of positive RSV and influenza A viral diagnosis in the OM-85 BV-treated group (75 children) and in the placebo group (70 children).

The extensive search for viruses by both direct rapid diagnostic tests and cultivation techniques permitted occasionally to detect more than one virus in the same sample. Table 3 gives the details of the mixed viral infections during the winters of 1992–1993 and 1993–1994. The viruses detected in mixed infections are those which largely spread and provoked outbreaks.

Results of the Combined Clinical - Virologic Study

During the winter season of 1989–1990 a large clinical trial evaluating the efficacy and the safety of OM-85 BV in children attending DCCs was conducted. A total of 423 children have been enrolled, 213 in the placebo group and 210 in the verum group. In summary, it could be demonstrated that OM-85 BV had a protective effect in significantly reducing the risk of recurrent infections of the upper respiratory tract during the treatment period. The complete results have recently been published [14].

In parallel to this clinical trial a preliminary virologic study was performed in order to assess the potential benefit of OM-85 BV on the viral carrier status of the participating children. Twenty-seven DCCs accepted to participate in this complementary study including 145 children (75 in the verum group, 70 in the placebo group). Eleven centers reported a total of 17 outbreaks of respiratory infections involving 36 children (19 in the verum group, 17 in the placebo group). Epidemics were due to RSV (n = 5), influenza A (n = 2), adenovirus (n = 2), enterovirus (n = 1) and undetermined (n = 7) (table 2) [15].

RSV was isolated 10 times in the placebo group, but only 5 times in the treated group ($p < 0.05$) and influenza A virus was present in 4 children in the placebo group, but only in 1 infant in the verum group giving a total of 14 positive virologic results in the placebo group versus 6 in the verum group ($p < 0.05$; fig. 5). Conversely, only 5 children in the placebo group had a negative virologic culture, compared to 11 in the verum group. Despite the small numbers of children investigated for their virologic status during respiratory infectious outbreaks, there was a statistically significant difference in the prevalence of virus carriers in favor of the children treated with OM-85 BV.

Discussion

The epidemiological pattern of the viruses responsible for outbreaks of respiratory infections varies from one winter to another. Furthermore, the DCCs only partly reflect the epidemiology of the respiratory viruses occurring in the general population. For this reason, it seemed interesting to compare the respective frequency of the viruses isolated in children attending DCCs with those from children of the same age but who attended a general or pediatric practice.

The alert net-work called 'Groupe Régional d'Observation de la Grippe' (GROG), founded in 1987, implemented an epidemiological survey including all patients, whatever their age, presenting an influenza-like disease (i.e. fever more than 38.5°C, sudden onset, acute infection of the upper or lower respiratory tract) from October

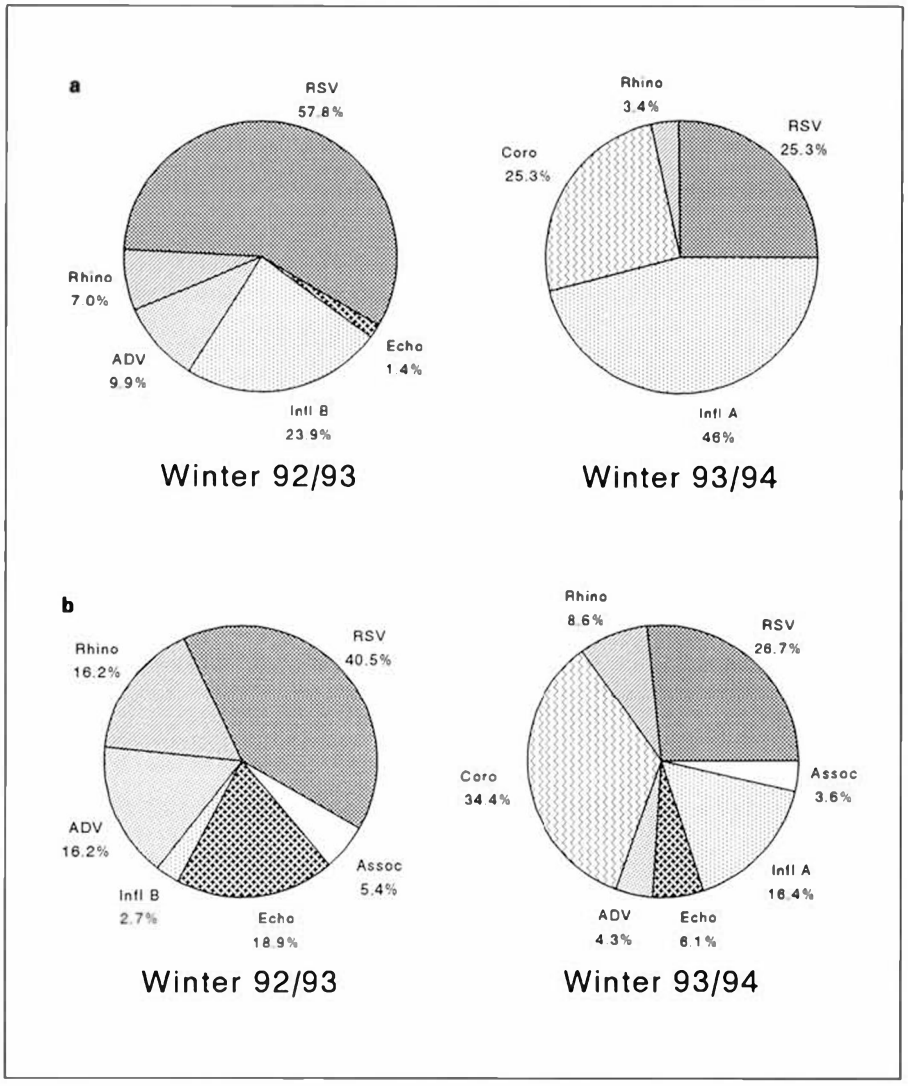

Fig. 6. Comparison of the relative proportion of individual viral diagnosis between children <3 years attending an outpatient facility (**a**) and children attending DCCs (**b**) (1992–1993 and 1993–1994).

to April. These had a nasal swab sampling for viral investigation according to the same procedure as for DCC-attending children [16].

The results for the children aged <3 years are summarized in figure 6. The most striking differences were:

(1) The high frequency of influenza B in general practice early 1993 as compared to only 1 case diagnosed in a DCC, whereas it was nil in the winter of 1993–1994.

(2) The higher frequency of influenza A H3N2 in general practice than in DCCs. Influenza A H3N2 was even more frequent than RSV infections in general practice which contrasts to what was observed in the DCCs, but it was not identified in 1992–1993 in any of the settings.

(3) Significant outbreaks of echovirus and rhinovirus infections were observed exclusively in DCCs, and the presence of several viruses in the same sample proved the large diffusion of these viruses in DCC.

These observations warrant some comments. Every winter, 30–50% of the epidemics of respiratory diseases are caused by RSV. However, during the season of 1993–1994, the spread of RSV was clearly less important than in the previous winters, and represented only 7 out of 42 (17%) epidemic events (table 2). RSV infections did not seem more frequent in DCCs than in general practice and the lower prevalence of RSV infections in the winter of 1993–1994 as compared to 1992–1993 was observed in both populations. There is a close correlation between the incidence of RSV in DCCs and the presence of the virus in the general population. The percentages of RSV isolated in both populations were not significantly different,

both during the high and the low epidemic season. In 1972, Loda et al. [17] have already published such an observation while following 39 children for 40 months.

The other respiratory viruses show some particular characteristics with variations from one season to another and interesting differences between the findings in DCCs and in general or pediatric practice.

The epidemiological pattern of infections due to influenza viruses A H1N1, A H3N2 and B revealed quite a different pattern in children attending DCCs as compared to the general population. Influenza A H3N2 was responsible for epidemic outbreaks of respiratory infections in 1989–1990 and again in 1993–1994, first in the general population and only later in DCCs. In spring 1993, influenza A H3N2 (a new variant) was spreading at a subepidemic level in the general population, but caused no epidemic in DCCs. The percentage of influenza A H3N2 viruses isolated from children in DCCs was significantly lower than the percentage of those isolated from children staying at home. While an influenza B epidemic was spreading in the general population in early spring 1993, the virus could be isolated only in one single child in a DCC.

We have a limited experience with the coronaviruses because an adequate monoclonal antibody has been made available only recently. In 1991–1992, an outbreak of coronaviruses representing 20% of all the positive respiratory viral samples was reported in Lyon, the capital of the Rhône Département [18, 19]. Clinically, these infections presented with fever, rhinitis and cough. In 1992–1993, coronavirus was never diagnosed in our laboratory, nor was it diagnosed in DCCs or in the general population. During the winter of 1993–1994, however, a large outbreak occurred, and coronavirus has been the predominant virus this last winter in DCCs. At the same time, influenza A H3N2 and RSV were frequently isolated. It is possible that DCCs favor the spread of coronaviruses: we have observed recurrent epidemic events caused by this particular virus in 3 DCCs, whereas relapsing epidemics caused by RSV or influenza A were observed in 1 DCC for each of the agents only.

Other respiratory viruses, i.e. rhinoviruses, adenoviruses and enteroviruses (echo), were responsible for epidemics in DCCs, whereas they were rarely isolated in children cared for at home.

Mixed viral infections in children have rarely been described when using classical virological and serological tests. The development of reagents of high specificity and avidity and the establishment of sensitive and easy-to-perform tests allowed us to confirm that various viruses can be present in one and the same respiratory sample. Such 'mixed' infections were particularly frequent during this last winter (1993–1994), in children attending DCCs (3.7%), in hospitalized children [3.4%, ref. 16] and in children staying at home [2.3%, ref. 16]. It is not possible, to date, to say whether 'mixed' viral infections are clinically more severe than infections due to a unique virus, nor is it possible to assume that DCC attendance favors such multiple infections.

The risk factors of DCC attendance are: the collective life and the resulting crowding, the exploratory behavior of children, the resistance of the viruses in the environment, the prolonged excretion of these viruses including through the feces and possibly the general hygiene conditions. In our observations, the viral respiratory infections in DCCs did not precede the outbreaks in the general population, except in 2 cases due to echovirus and rhinoviruses. The role of children attending DCCs as a reservoir for contaminating their parents and the population could not be confirmed in this study.

In the light of these epidemiological data, it is obvious that a prophylactic control of respiratory virus excretion would be highly desirable. OM-85 BV has been shown to significantly reduce the number of recurrent respiratory infections in children [20] and adults [21]. Given the fact that the bacterial extract induces a nonspecific stimulation of the mucosal immune defense mechanisms [22], it can be hypothesized that this protection is also conferred to viral infections of the upper respiratory tract in children. Despite the small numbers of children investigated in this study for their virologic status during respiratory infectious outbreaks, there was a statistically significant difference in the prevalence of virus carriers in favor of the children treated with OM-85 BV. These results corroborate the clinical findings. In conclusion, the preliminary data with OM-85 BV demonstrate a significantly reduced incidence of positive cultures for RSV and influenza A as compared to placebo and indicate a new and potentially very useful approach towards controlling recurrent outbreaks of respiratory illnesses in DCCs.

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