



Viral Aetiology of Severe Acute Respiratory Infections in Hospitalised Adult Patients in Abidjan, Côte d'Ivoire

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Authors' contributions

This work was carried out in collaboration among all authors. Author JAD designed the study, performed the statistical analysis, wrote the protocol and the first draft of the manuscript. Authors AK and HK investigated the field outbreak and collected samples required for diagnosis and described the disease epidemiology. Authors SMASN and MAN conducted the laboratory work. Authors HK and AKN managed the analyses of the study. Author HFK collected the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aims: Pneumonia is a leading cause of morbidity and a significant cause of mortality worldwide. Although information is available on pneumonia in children, the incidence in adults in many parts of Africa including Côte d'Ivoire is unknown. Knowledge of local etiologic agents of pneumonia is critical for making reasonable decisions about treatment as differences in etiology may result in poor response to therapy chosen to cover common pathogenic microbes in studies done in high countries of income.

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The objective of this study was to identify the viral etiology of pneumonia in adult patients with pneumonia in Abidjan, Côte d'Ivoire.

Study Design: This is a prospective experimental study conducted on the basis of the successive recruitment of patients admitted to hospital for severe pulmonary interstitial pneumonitis confirmed by radio or CT scan of the thorax.

Place and Duration of Study: Pneumophtisiology department (PPH) of the University Hospital Center of Cocody (Côte d'Ivoire) and laboratory of Bacteriology- Virology of Pasteur Institut of Côte d'Ivoire, between February 2016 and October 2017.

Methodology: Among all admitted patients in the unit of pneumophtisiology (PPH) of the hospital University, 90 patients aged at least 18 years were pre-included. A total of 33 bronchoalveolar lavage fluid (BAL) samples from adults suspected of pneumonia were analyzed. The viruses were identified by the real-time multiplex reverse polymerase chain reaction (RT-PCR).

Results: Of the 33 BAL samples tested, 18.2% (6/33) viral agents were detected. Parainfluenza-3 PV-3 was the most prevalent virus (57.1%, 4/7), followed by coronavirus OC43 (14.3%, 1/7), coronavirus HKUI (14.3%, 1/7).) and human rhinovirus (14.3%, 1/7).

A virus and virus association was detected, which was PV-3 associated with coronavirus HKUI (14.3%, 1/7).

Conclusion: The viral etiology of pneumonia is not very frequent in Côte d'Ivoire.

Keywords: Pneumonia; respiratory viruses; adults; real-time multiplex PCR.

ABBREVIATIONS

ARI : Acute respiratory infections.

BAL : Bronchoalveolar lavage fluid.

1. INTRODUCTION

Acute respiratory infections (ARI) are very common ubiquitous conditions, affecting both adults and children [1]. The infectious etiologies are extremely varied. Among them, bacteria and viruses are most frequent cause of infection, viruses are responsible for most severe form of infection [1]. Thus, viral respiratory infections are a significant aspect of bronchopulmonary infections [1]. Pneumonia is the most severe form of all lower respiratory tract infection [2]. It is a common infectious disease with an estimated incidence of 2 to 11 cases per 1,000 adults in developed countries and a mortality rate of 2% to 14% [3,4]. There is a dearth of information on the prevalence of respiratory tract infection caused by viruses as they draw attention only when there is a flare up of infection. However, viruses have been reported to be responsible for 15-40% of RTI [5]. In addition, frequency of viral infection decreases in healthy adults but increases significantly in the elderly [6].

Of the 13-50% of diagnosed cases of community-acquired pneumonia, viruses have been reported as the second most frequently isolated cause [7].

In addition, the causative agents are in most cases of the viral type with among them:

influenza and para-influenza viruses, respiratory syncytial virus, rhinoviruses, coronaviruses, and adenoviruses [8].

Since molecular biology methods have complemented conventional methods such as viral culture and immunoassays; the diagnosis of viral respiratory infections has not only increased in sensitivity, specificity and rapidity but has also made it possible to detect new virus subtypes [9].

Viral diagnostic methods have evolved significantly with the advent of molecular biology techniques and more specifically so-called "multiplex" molecular tests for the simultaneous detection of a large number of infectious agents [1]. Indeed, various techniques derived from the PCR (Polymerase Chain Reaction) have many advantages: the real-time PCR or RT-PCR ("reverse transcription polymerase chain reaction") allows not only specific but also quantitative detection of viral nucleic acids (DNA or RNA) and multiple techniques can co-detect several different viruses in a single reaction in the presence of signs of respiratory infection [9]. This method is therefore ideal for the rapid detection of a viral origin with panels that can include up to more than 12 different viruses and to identify viral co-infections and study their clinical impact [9].

The purpose of this study is to determine infections of viral origins associated with pneumonia in adult patients hospitalized at the University Hospital Center of Cocody Abidjan, Côte d'Ivoire.

2. MATERIALS AND METHODS

2.1 Description of the Study

This is a prospective experimental study conducted on the basis of the successive recruitment of patients admitted to hospital for severe pulmonary interstitial pneumonitis confirmed by radio or CT scan of the thorax, between February 2016 and October 2017. Among all admitted patients in the unit of pneumophthysiology (PPH) of the hospital University, 90 patients aged at least 18 years were pre-included. The analysed samples were composed of blood, serum, sputum of patients suspected of having pneumonia. After this biological assessment (Tuberculosis, serology HIV, glycemic, creatinin, transaminase, CRP), 57 patients were excluded at a rate of 63.3% (Fig. 1). Inclusion criteria are inpatients, of both sexes, aged 18 years and older with severe febrile alveolo-interstitial pneumonitis confirmed by standard chest radiograph and the negativity of tuberculosis bacilli sputum. The exclusion criteria were the patients detected tuberculous and those refusing to do fibroscopy.

Only 33 (36.7%) patients were included and underwent fibroscopy to obtain bronchoalveolar lavage fluid (BAL). BAL was performed during fibroscopy by instillation and aspiration of saline into the nasal cavity in sub segmental bronchi. Thirty three LBAs samples were collected in a sterile disposable container and sent to the Bacteriology-Virology laboratory in transport containers containing cold accumulators, within one hour for virus detection.

2.2 Extraction of RNA and DNA

Bronchoalveolar lavage samples were placed in Eppendorf tubes after specimens collection. Thus, total viral nucleic acids (DNA or RNA) were extracted from 140 µL of each clinical sample of BAL using the QIAamp® viral RNA mini kit, QIAGEN for the extraction of RNA viruses and the QIAamp® DNA mini kit, QIAGEN for that of viruses. The DNA / RNA was eluted with 60 µL of AVE elution buffer supplied with the kit and stored at -80°C until use.

2.3 Amplification and Molecular Detection of Viruses by Real-time Multiplex PCR

Samples were analysed using the Super script®III platinum® One-step qRT-PCR system

detection kit (Van Allen Way Carlsbad CA 92008, USA) for simultaneous detection of respiratory viruses. Two DNA viruses (Adenovirus and Bocavirus) and 14 RNA viruses were amplified and detected using a real-time thermal cycler: ABI® 7500 FAST. This kit uses a 5-tube multiplex PCR technique for the simultaneous detection and identification of viruses. The primers and probes used were recorded in Table 1.

For viruses with RNA, the amplification conditions consisted of a step of reverse transcription of the RNA into DNA for 30 min at 50°C followed by an initial denaturation and activation step the Taq polymerase for 2 min at 95°C. A second step of the amplification was performed in the same conditions by 45 denaturation cycles at 95°C for 15 s, hybridization at 55°C for 30 s and extension at 55°C for 30 s. Finally, a terminal extension at 4°C for 10 min.

For viruses with ADN, the amplification conditions consisted of a step of pre-activation of the enzyme for 2 min at 50°C, followed by an initial denaturation and activation step the Taq polymerase for 10 min at 95°C. A second step of the amplification was performed in the same conditions by 45 denaturation cycles at 95°C for 15 s, hybridization at 60°C for 1 min and extension at 60°C for 1 min.

2.4 Statistical Analysis

Data entry and description were performed using Epi-info software version 7.2.0.1. These data were then transcribed into an Excel database making their analysis easier. Statistical tests were interpreted at the significance level corresponding to an alpha risk of 5%. Statistical analyzes were analyzed using the STATA version 15.0 software. Thus, the quantitative variables were expressed as mean and standard deviations of the mean, and the qualitative variables as numbers and percentages. The Chi2 statistical test was used for proportion comparisons with a significance threshold *P* of 0.05.

3. RESULTS

3.1 Age Distribution by Gender

The age of patients ranges from 19 to 68 years, with an average of 39.6 years. The incidence of

pneumonia according to the age of the patients is as follows: 19-30 years (27.3%, 9/33), 30-40 years (48.5%, 16/33), 40-50 years (6.1%, 2/33) and over 50 years (18.1%, 6/33) Table 2.

3.2 Patient Distribution by Gender

Based on inclusion criteria, 33/90 patients continued the study. Of the 33 patients suspected of having pneumonia, our series included 16 women or 48.5% of cases and 17 men or 51.5% of cases. The sex ratio H / F was 1.1.

3.3 Period of Recruitment of Patients According to the Seasons

The breakdown of patients included according to the season is as follows: from March to June (45.5%, 15/33) followed by the season from November to February (24.2%, 8/33), then from September to October (24.2%, 8/33) and finally the season from July to August (6.1%, 2/33) Fig. 2.

3.4 Detection and Identification of Respiratory Viruses by Real-time Multiplex PCR

Detection of respiratory viruses from a total of 33 BAL samples resulted in 7 positive cases (21.2%) and 26 negative cases (78.8%). Real-time PCR performed on 33 BAL samples revealed the presence of the following viruses: coronavirus OC43 (14.3%, 1/7), parainfluenza-3 PV-3 (42.9%, 3/7), rhinovirus human (14.3%, 1/7). A viral coinfection has also been demonstrated in these BAL samples. Parainfluenza-3 (PV-3) was associated with coronavirus HKUI (14.3%, 1/7). The most incriminated virus in this study was the parainfluenza-3 virus (57.1%, 4/7).

3.5 Distribution of Viruses According to Age

Seven viruses were detected in this study. In patients aged between 19-30 years, a total of 3 isolates including 2 types of virus (42.8%, 3/7) were identified. These included the following viruses: parainfluenza-3 PV-3 (28.6%, 2/7) and coronavirus OC43 (14.3%, 1/7); in patients aged between 30-40 years, 2 viruses (28.6%, 2/7), parainfluenza-3 PV-3 (14.3%, 1/7), and viral infection parainfluenza-3 (PV-3) associated with coronavirus HKUI (14.3%, 1/7), in those having

40-50 years, rhinovirus was found (14.3%, 1/7) and finally it has not been detected in adults over the age of 50 with no virus (Fig. 3).

There is no significant difference between age and virus detection because the calculated probability is higher ($P = 0.136 > P = 0.05$).

3.6 Classification of Viruses by Sex

Of the seven viruses, two men were detected positive for viruses: 1 coinfection Parainfluenza-3 (PV-3) + Coronavirus HKUI and 1 Parainfluenza-3 (PV-3). These two men are 40 years old and 27 years old.

Four women were detected positive for the following viruses: 1 Coronavirus OC43, 2 Parainfluenza-3 (PV-3) and 1 human Rhinovirus. These women were aged 24, 30, 33 and 42 years old Fig. 4.

3.7 Virus Detection According to the Seasons

In this study, the distribution of viruses according to the seasons revealed the following results: the season of March-June PV-3 (28.6%, 2/7), coronavirus OC43 (14.3%, 1/7), PV-3 + HKUI (14.3%, 1/7); the July-August season: no virus detected (0%, 0/7); the September-October: human rhinovirus season (14.3%, 1/7) and November-February PV-3 (14.3%, 1/7) (Fig. 5). Viral detection has seen a peak in the March-June season.

The age distribution of patients detected positive for respiratory viruses was very different between the four seasons. During the March-June season, most of the patients affected were young adults aged 19-30 (PV-3, 28.6%, OC43, 14.3%). In the March-June period, patients aged 30-40 years (PV-3 + HKUI, 14.3%) as well in the period of September-October and November-February, patients aged 40-50 years (rhinovirus, 14.3%) and people aged 30-40 (PV-3, 14.3%) were very little affected respectively.

4. DISCUSSION

The objective of this study was to determine the viral etiology of pneumonia in Côte d'Ivoire particularly in Abidjan. During the study period (2016-2017), we included patients whose age was greater than or equal to 18 years. Thus, the age between 30-40 years was strongly represented with a rate of 48.5%. This result is contrary to that obtained in the Taqarort study in

which the study population aged 30-39 was the least represented with a rate of 9.8% [10]. The age group over 50 represents 18.1% of our study. This rate is close to that published by Taqarort which is 17.1%. This may be due to the fact that age is a risk factor that is independent of the occurrence of pneumonia [10] and that the frequency of hospitalization for severe pneumonia also increases with age, as well as mortality [11] Table 2.

Moreover in our series, the study showed that 51.5% of men were the most involved in the occurrence of viral pneumonia than women (48.5%). Our results are in agreement with those of the literature which observes a male predominance in other studies, with varying proportions [12]. This could be explained by the fact that risk factors for pneumonia such as smoking and alcoholism are found in men. This male predominance was also observed in the other studies, with varying proportions. Our results are similar to those of Barouhiel [13] who found men proportions of 52% and women 48%. Our results are also consistent with those of Horo et al. [14] who found that male dominance is the rule in community-acquired pneumonia [14,15]. In addition, the determination of the rate of patients recruited for pneumonia in this study revealed a high rate in the month of March to June which amounted to 45,5%. This rate is higher than previous studies reporting that

pneumonia occurred mainly in winter and then in autumn with a rate of 39% [16].

This result could be explained by the fact that in Côte d'Ivoire we have four (4) seasons: a big rainy season from March to June, a short dry season from July to August, a short rainy season from September to October and finally a long dry season from November to February [17]. The high number of patients recruited this season could be due to the fact that during the months of March to June we are in the rainy season and it is very cold. According to the literature, pneumonia can be observed throughout the year with maximum frequency in winter because cold seasons are conducive to respiratory infections [10] Fig. 2. Also, the distribution of viruses according to the age groups according to our study made it possible to detect that the majority of our patients are located in the slice between 30-40 years with a rate of 48,5%. In the series of Bouaïti, most of his patients were over 60 years old. Our results are different from those of the Bouaïti study, which detected a low rate of 10.3% in the 30-40 age group [18]. This could be explained in part by the phenomenon of demographic aging, which is becoming more evident in our country than in previous years. Also, this can be explained by the fact that in developing countries like Côte d'Ivoire, the age of predilection of this pathology is between 20 and 49 years old with an average age ranging from

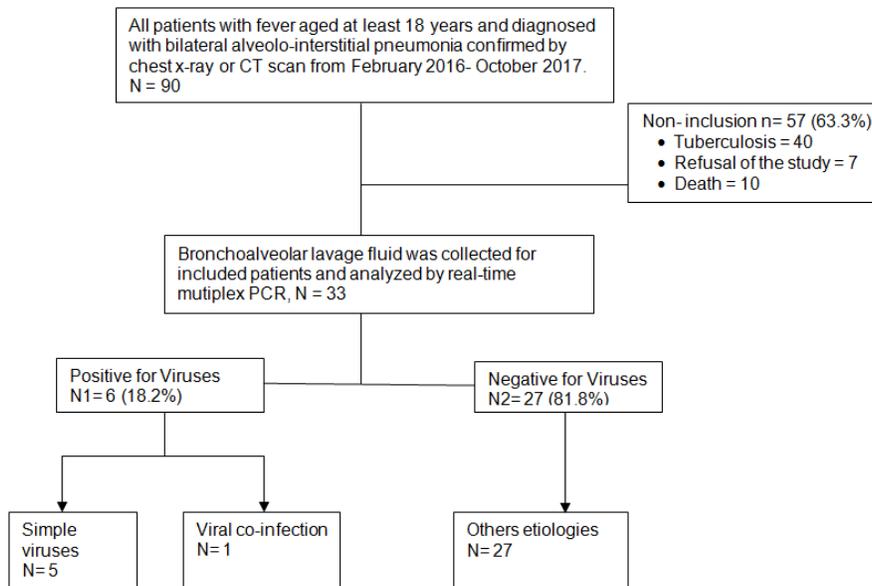


Fig. 1. Recruitment chart of adult pneumonia patients and detection of viral etiology by real-time multiplex PCR

Table 1. Primers and probes used for the detection of respiratory viruses

Multiplex	Viruses	Sequence (5'- 3')
1	PIV-1	Fwd: GTTGTC AATGTCTTAATTCGTATCAATAATT Rev: GTAGCCTMCCTTCGGCACCTAA Pr: (FAM)-TAGGCCAAAGATTGTTGTGCGAGACTATTCCAA-(TAMRA)
	PIV-2	Fwd: GCATTTCCAATCTTCAGGACTATGA Rev: ACCTCCTGGTATAGCAGTGAAGTGAAC Pr: (CY5)-CCATTTACCTAAGTGATGGAATCAATCGCAAA-(BHQ-2)
	PIV-3	Fwd: TGATGAAAGATCAGATTATGCATATC Rev: CCGCGACACCCAGTTGTG Pr: (ROX)-TGGACCAGGGATATACTACAAAGGCCAAAAT-(BHQ-2)
2	COV-OC43	Fwd : CGATGAGGCTATTCCGACTAGGT Rev : CCTTCCTGAGCCTTAATATAGTAACC Pr : (ROX)-TCCGCCTGGCACGGTACTCCCT-(BHQ-2)
	HRV	Fwd : AGTCCTCCGGCCCTGAAT Rev : ACACGGACACCCAAAGTAGT Pr : (CY5)-TGAGCAATTGTGGATGGGA-(BHQ-2)
	FLUB	Fwd : AAATACGGTGGATTAATAAAAAGCAA Rev : CCAGCAATAGCTCCGAAGCAA Pr : (JOE)-CACCCATATTGGGCAATTTCTATGGC-(BHQ-1)
3	HMPV	Fwd : ATGTCTCTTCAAGGGATTCACCT Rev : AMAGYGTATTTCTTGTGCAATGATGA Pr : (JOE)-CATGCTATATTAAGAGTCTCARTAC-(BHQ-1)
	VRS	Fwd : GCAAATATGCAAACATACGTGAACA Rev : GCACCCATATTGTWAGTGATGCA Pr : (ROX)-CTTCACGAAGGCTCCACATACACAGCWG-(BHQ-2)
	FLUA	Fwd : CTTCTAACCGAGGTCGAAACG Rev : AGGGCATTGTTGGACAAAACGCTCTA Pr : (FAM)-CCTCAAAGCCGAGATCGCGCA-(BHQ-1)
4	COV-229E	Fwd : CAGTCAAATGGGCTGATGCA Rev : AAAGGGCTATAAAGAGAATAAGGTATTCT Pr : (FAM)-CCCTGACGACCACGTTGTGGTTCA-(TAMRA)
	COV-HKUI	Fwd : CTTGCGAATGAATGTGCT Rev : TTGCATCACCCTGCTAGTACCAC Pr : (CY5)-TGTGTGGCGGTTGCTATTATGTTAAGCCTG-(BHQ-2)
	COV-NL63	Fwd : ACCTAATAAGCCTCTTTCTCAACCC Rev : GACCAAAGCACTGAATAACATTTTCC Pr : (JOE)-AACACGATTCCAACGAGGTTTCTTCAACTGAG-(BHQ-1)
5	ADV	Fwd : GCCACGGTGGGGTTTCTAAACTT Rev : GCCCCAGTGGTCTTACATGCACATC Pr : (FAM)-TGCACCAGACCCGGGCTCAGGTA CTCCGA-(TAMRA)
	BOV	Fwd : GCACAGCCACGTGACGAA Rev : TGGACTCCCTTTTCTTTGTAGGA Pr : (JOE)-TGAGCTCAGGGAATATGAAAGACAAGCATCG-(BHQ-1)

Table 2. Distribution of pneumonia cases by age

Age (year)	n	%
19- 30	9	27,3
30- 40	16	48,5
40- 50	2	6,1
>50	6	18,1
Total	33	100

35.12 to 42.05 years old [19,14]. Certainly the large population at a very young age, the precarious living conditions and the HIV infection are the factors that explain this observation [20]. On the other hand, our results are similar to those of Dhaimi whose studied population was younger [21]. Also, the oldest patients (> 50 years) in our study are less represented with a rate of 18.1%. This result is approximately similar to other studies in which 60-69 year olds were

reached at a rate of 17.1% [10] and 15% according to the Dhaimi study, [21] Fig. 3.

The study showed that the detection rate of viruses in women is higher (4/7, 57.1%) than men (3/7, 42.9%). This results are different to those of Barouhiel [13] who found men proportions of 52% and women 48% Fig. 4.

This study revealed a viral etiology in 7 cases (21.2%) of 33 pneumonia patients. These results approximate those of other studies in which viral infection rates in pneumonia patients increased from 23-56% [22,23,24]. This difference would

probably be due to the different methods chosen and the distinctions of different regions and populations. So this difference could be explained by the difference in climate and season in the countries where the studies would be conducted. In tropical environments, the incidence is highest during the rainy season. Indeed, the incidence of infections varies with the season; the frequency is higher in winter and spring [25]. Viral detection showed a peak in the March-June season (28.6%) because the season from March to June is a cold period Fig. 5.

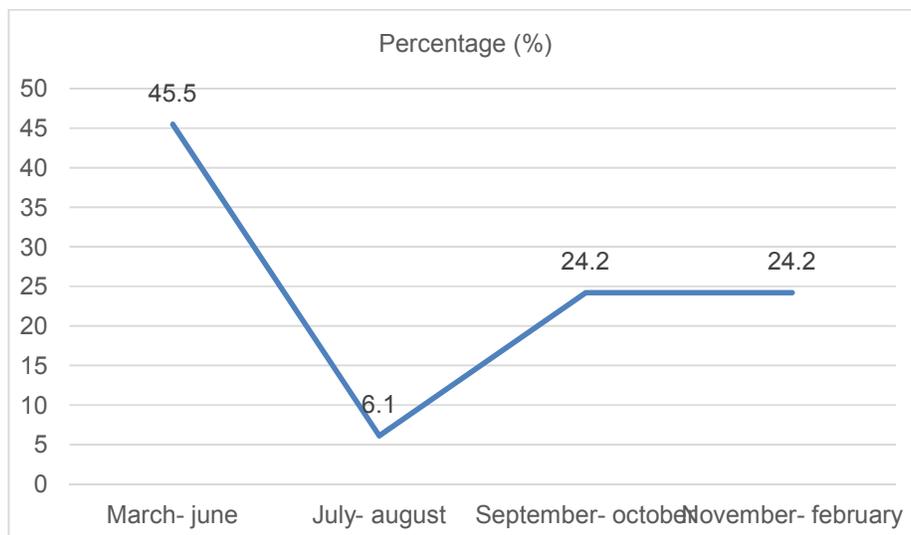


Fig. 2. Recruitment of patient according to the seasons

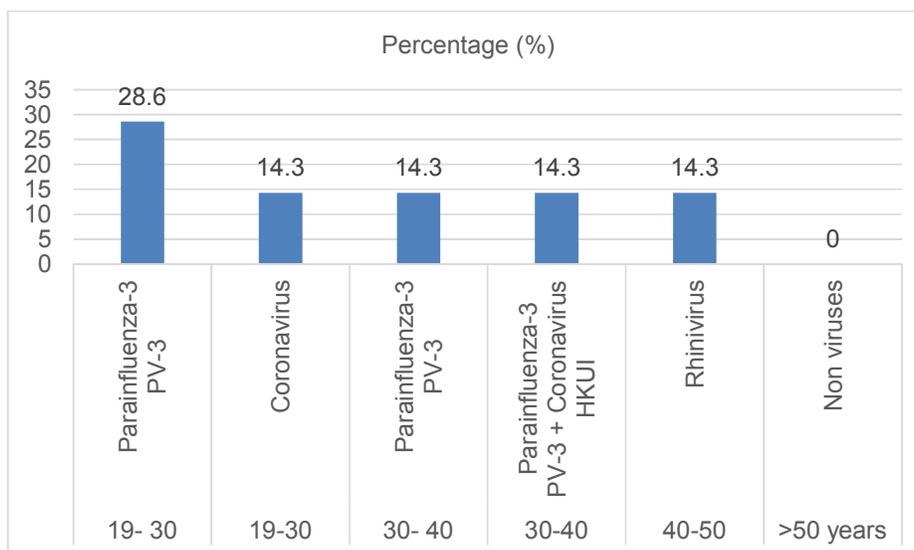


Fig. 3. Distribution of viruses by season

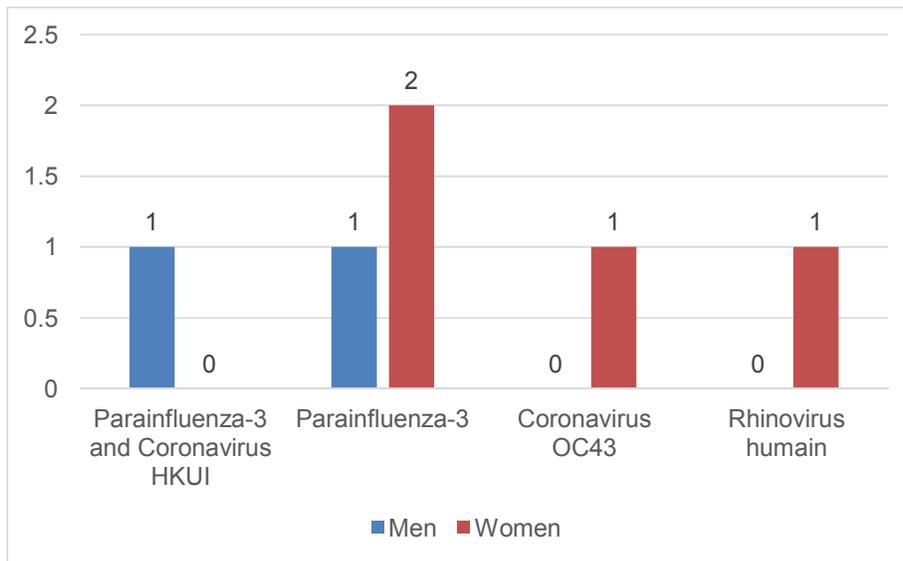


Fig. 4. Distribution of viruses by gender

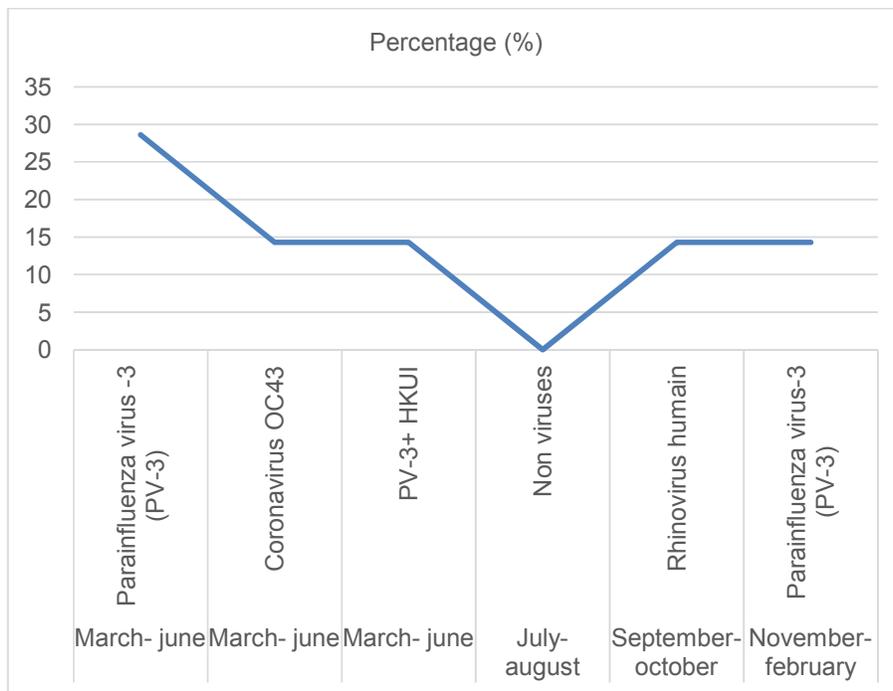


Fig. 5. Classification of viruses according to the seasons

PCR in this study revealed four types of viruses including OC43 coronavirus, Coronavirus HKUI, human rhinovirus and Para-influenza virus. The most incriminated virus in patients with pneumonia was Parainfluenza-3 (57.1%, 4/7). In fact, according to a study conducted in Lorraine, the PIV-3 subtype was the most frequently found subtype (62.7%)

compared to the other PIV-1 subtypes (25.3%), PIV-2 (7.3%) and PIV-4 (4.6%) [26]. Our results are consistent with those of Thomazelli et al. [27] who found a 57.7% positivity rate for parainfluenza-3 virus. This may be due to the fact that PIV-3 subtype infections occur in the spring and especially in the summer of each year [28].

5. CONCLUSION

The study of a series of 33 cases of hospitalized pneumonia from 2016 to 2017 at the University Hospital Center of Cocody, Abidjan, Côte d'Ivoire, allowed us to note that this pathology is not frequent enough. The study population was predominantly 19-30 years old, male and of low socio-economic status. Hospital recruitment explains the significant frequency of the disease during cold seasons. The occurrence of acute pneumonia is most commonly seen in individuals with a particular field.

Thus, the search for viruses by real-time PCR in the bronchoalveolar lavage fluid gave a low detection rate of 18.2% with four (4) types of virus for a range of ten (10) that we offer the viral detection kit. These are Coronavirus OC43, Parainfluenza-3 PV-3, Human Rhinovirus and Coronavirus HKU1. These respiratory viruses cause seasonal infections in both children and adults, resulting in a wide range of clinical syndromes such as a common cold, laryngitis, bronchiolitis, but also more severe conditions such as pneumonia.

CONSENT

All authors declare that written informed consent was obtained from all the patient for the study.

ETHICAL APPROVAL

This study was approved by the national ethics committee according to decision n°31 / msls / cnfr-dkn of 23 june 2015.

All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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