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First Report of Human Parainfluenza Viruses among Patients with Respiratory Symptoms in Khartoum State, Sudan

Awadalkareem Y. Awadalkareem[1,2], Abdel Rahim M El Hussein[2], Naglaa M Ahmed[1], Mohamed O Mustafa[2], Isam M. Elkhidir[2], Mutaz A Elsir[2], Ibrahim M Salih[3], Khalid A. Enan[2,3]

[1] AL Neelain University, Faculty of Medical Laboratory Sciences, Medical Microbiology Department, Khartoum, Sudan,
[2] Central Laboratory, Ministry of High Education and Scientific Research, Khartoum, Sudan.
[3]Preventive Reference Laboratory, Department of Health Protection & Communicable Diseases Control, Ministry of Public Health, Doha, Qatar

Corresponding Author: Khalid A Enan, Email: khalid.enan@gmail.com

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ABSTRACT

Background: This study was carried out to detect human Parainfluenza virus 'HPIV' RNA in patients with respiratory infections in Khartoum state in order to determine the circulating types of Parainfluenza virus 'in Khartoum state, Sudan. In addition, the study aimed to locally establish the diagnosis of parainfluenza virus using of RT-PCR.

Methods: The study was conducted in Khartoum state Hospitals during the period from January 2019 to February 2021. Nasopharyngeal swabs samples were collected from 149 patients with respiratory symptoms. Samples were processed for RNA extraction and virus isolation. Extracted RNA was subjected to RT-PCR for the detection of Parainfluenza virus RNA and to further determine Parainfluenza virus serotypes. Positive samples were inoculated in chicken embryonated egg to isolate the virus.

Results: Out of 149 samples 13 samples (8.7%) were positive and 136 samples (91.3%) were negative for parainfluenza virus RNA using RT-PCR. Parainfluenza types 1 and 2 were not detected while parainfluenza-3 was detected in 8 sample (5.4%) and parainfluenza-4 was positive in 5 samples (3.4%). Among PIV RNA positive samples (n=13); no virus was isolated using embryonated eggs inoculation.

Conclusion: Existence and incidence of human parainfluenza virus and its types in Sudan was documented through detection of Human PIV(HPIV) RNA in respiratory samples. Moreover, the HPIV detection using RT-PCR was established.

Keywords: Human Parainfluenza, Serotypes, RT-PCR, Emberyonated egg.

INTRODUCTION

Parainfluenza viruses (PIV) were first discovered in 1950. They are enveloped, non-segmented, negative single-stranded RNA viruses that belong to the family Paramyxoviridae. PIVs are genetically and antigenically divided into four types: PIV-1, PIV-2, PIV-3, and PIV-4, each with different genetic and antigenic features [1]. Serotype 4 can be further subdivided into two antigenic subtypes, PIV-4A and PIV-4B [2].

PIVs are a cause of community-acquired pneumonia in healthy individuals and can infect individuals of any age group [3, 4]. The majority of PIV-infected patients are treated in outpatient clinics, yet PIV infections are one of the most common causes of respiratory diseases leading to hospitalization [3,5,6]. Among immunocompromised patients, PIV, especially type 3, has been associated with serious outcomes and complications [7]. PIV can also be clinically significant in ICU and PICU patients [8, 9]. However, not much is known about the burden of PIV infections among ICU and PICU patients. Up to now, no specific antiviral drugs or effective vaccine are available despite progress made in these fields recently [10, 11]. HPIV are common community-acquired respiratory pathogens without ethnic, socioeconomic, gender, age, or geographic boundaries. Many factors have been found that predispose to these infections, including malnutrition, overcrowding, vitamin A deficiency, lack of breastfeeding, and environmental smoke or toxins [12-16]. There are more than 5 million lower respiratory tract infections (LRI) each year in the United States in children younger than 5 years. HPIV-1 to HPIV-3 have been found in as many as one-third of these infections [17-19]. In addition, HPIVs cause upper respiratory tract infections (URI) in infants, children, and adults and, to a lesser degree, LRI in the immunocompromised, or those with chronic diseases (e.g., heart and lung disease and asthma) and the elderly. Little is known about HPIV-4; young infants and children are clearly infected by this virus, but it is seldom isolated. Serologic surveys have indicated that most children between 6 and 10 years of age have evidence of past infection [1]. Biennial fall epidemics are the hallmark of HPIV-1 and occur worldwide [11-21]. Reports from the United States have suggested that a minimum of 50% of all croup cases are caused by this virus. During each HPIV-1 epidemic, an estimated 18,000 to 35,000 U.S. children younger than 5 years are hospitalized [22]. Lower respiratory tract infections are considered the principal cause of hospitalization and death in certain groups of people [3, 23, 24]. The clinical symptoms of respiratory tract infections include croup, bronchitis, bronchiolitis, and pneumonia; however, some clinical manifestations are more related to individual HPIVs. HPIV1 and HPIV2 are most likely to cause croup, whereas HPIV3 and HPIV4 are more associated with bronchiolitis and pneumonia [25, 26].

The etiological diagnosis of HPIV infections cannot be based solely on clinical signs and symptoms because other pathogens can cause similar syndromes. The use of classic diagnostic methods, such as viral isolation and serology, can result in delays of several weeks before test results are available. Rapid diagnosis is thus desirable to assist the clinician in making therapeutic decisions and to prevent nosocomial infections [1, 27]. Direct antigen detection with respiratory specimens provides rapid results, but different methods such as immunofluorescence [22, 28, 29] or enzyme immunoassay have been reported to have variable sensitivities depending on the virus. Molecular techniques based on reverse transcription (RT)-PCR constitute another approach to rapid diagnosis with expected high sensitivity. RT-PCR assays have been applied to the detection of HPIV-1 and HPIV-3 [30-32] in monospecific assays or for the simultaneous amplification of HPIVs with other respiratory viruses [33-36]. An earlier study conducted by Sahar Essa et al. in Kuwait found that the most frequently isolated PIV type was HPIV-3 (71.8%), followed by HPIV-1 (23.1%). The least frequently isolated was HPIV-4 (5.1%), and HPIV-2 was not detected [6]. A longitudinal study conducted in Beijing reported that, from 81 HPIV RNA-positive cases, 10 were HPIV-1, 9 were HPIV-2, 55 were HPIV-3, and 7 were HPIV-4, with yield rates of 0.81%, 0.73%, 4.48%, and 0.57%, respectively [3]. In a retrospective study conducted in Atlanta, USA, HPIVs

were recovered from 32 of the 162 suitable samples; HPIV-1 was recovered from 21 (12.9%), HPIV-2 from 6 (3.7%), HPIV-3 from 2 (1.2%), and HPIV-4 from 1 (0.6%) [37].

MATERIALS & METHODS

Study approach:

A qualitative approach aimed to detect the Parainfluenza viruses 1, 2, 3 and 4 RNA among respiratory patients

Study area:

This study was conducted in different hospitals at Khartoum State, Sudan during the period from 2019 to 2021.

Study design:

This study is descriptive, hospital based cross- sectional study.

Data collection:

Ethical approval was obtained from the Central Laboratory ethical committee for collection and examination of the samples. The collected data included gender, date of sample collection, age, and place of sample collection. The obtained data were saved for statistical analysis.

Inclusion criteria:

Nasopharyngeal nasal and throat swab samples were collected from patients with common colds or flu like illnesses in Khartoum State, between January 2019-February 2021., They were only taken from individuals with symptoms of fever, cough, and fatigue of 3 days duration or less.

Specimens' collection:

A total of 149 Nasopharyngeal swabs were collected in transport medium from suspected patients. The patients included 88 males and 61 females (table 3)
that were categorized into three age groups; 0- less than1year; 1-less than 2years; and 4-5 years (Table 4). According to the respiratory clinical syndromes the patients were categorized into three groups, bronchiolitis, pneumonia and bronchopneumonia (table 5).
The samples were stored at -80°C for further uses. Transport medium consisted of Hanks balanced salt solution supplemented with 10% glycerol, 200 U of penicillin per ml, 200 mg of streptomycin per ml, 100 U of polymyxin B sulfate per ml, 250 mg of gentamicin per ml, and 50 U of nystatin per ml (all from ICN, Zoetermeer, The Netherlands).

RNA Extraction:

RNA was extracted using QIAamp viral RNA mini kit (Qiagen, Germany) according to manufacturer's instructions.

Performance of Reverse Transcriptase real-time PCR assay:

Real-time PCR was performed on the stored extracted RNA using one-step real-time PCR with parainfluenza real-time detection primers and probe [38] and SOLIScript 1-step Probe kit master mix following the manufacturer's instructions. The thermocycler amplification program for PIV detection was optimized as follows: reverse transcription step at 50°C for 15 minutes, followed by initial denaturation at 95°C for 10 minutes, then 45 cycles of denaturation at 95°C for 60 seconds and annealing at 60°C for 60 seconds.

1: PIV primer and probe sequences:

- Para influenza-1

Para-1 F	5'-ACCTACAAGGCAACAACATC-3'
Para-1 R	5'-CTTCCTGCTGGTGTGTTAAT-3'
Para-1 P	5'-6-FAM-CAAACGATTGCTGAATTTGGGA-BHQ-1-3'
	• Para influenza-2
Para-2 F	5'-ATCCAATCGATACTCGGAGGT-3'
Para-2 R	5'-TCTGGTTGTTTGGTTGTCCA-3'
Para-2 P	5'-6-FAM- TGATGGTGAGGACAGAATTGACAAC-BHQ-1-3'
Para-3 F	• Para influenza-3 5'-AAGATCTACAAGTTGGCAYAGCAA-3'
Para-3 R	5'-AATGTCCCCATGGACATTCAT-3'

Para-3 P	5'-HEX-TTCCTGGTCTTGATAGCACATTATGCCA-BHQ-1-3' • Para influenza-4
Para-4 F	5'-ACACYCAACAAATYAAAGGTTCAC-3'
Para-4 R	5'-TCCAGGRTCCATTATTTTCA-3'
Para-4 P	5'-HEX-TGCMACAATTGAGGGCTTAATYACRACA-BHQ-1-3'

Virus inoculation in Embryonated chicken eggs:

Only samples which tested positive for HPIV RNA by Real time were inoculated into allantoic sac of 9-11 day old embryonated eggs using 0.2 inoculum and the eggs were incubated at 37 °C for 4 days and candled daily. Eggs with dead embryos within 24 h of inoculation were discarded. Embryos that have died later than 24 h post-inoculation were refrigerated, and all eggs were chilled including the remaining eggs with viable embryos at 4 °C after the fourth day post inoculation. The allantoic fluids were harvested using 5 ml syringes with 18 G needles and the harvested allantoic fluid was stored at -20c for further use.

For confirmation, RNA was extracted from allantoic fluid and Real time PCR was performed for detecting parainfluenza virus RNA as prescribed above.

STATISTICAL ANALYSIS

The generated data was analyzed by using statistical package for the social sciences (SPSS version 23.0 software). A *p* value of ≤ 0.05 was considered significant.

RESULT

Frequency of parainfluenza RNA using RT-PCR assay:

Out of 149 samples 13 samples (8.7%) were positive and 136 samples (91.3%) were negative for HPIV RNA using RT-PCR. HPIV types 1 and 2 were not detected while HPIV -3 was detected in 8 sample (5.4%) and HPIV -4 was positive in 5 samples (3.4%). (Table.2).

Table.2: Frequency of parainfluenza RNA among patients with respiratory symptoms Khartoum state, Sudan 2019:

	HPIV -1	HPIV -2	HPIV -3	HPIV -4	Total
Positive	0 (0%)	0 (0%)	8 (5.4%)	5 (3.4%)	13 (8.7%)
Negative	149 (100%)	149 (100%)	141 (94.6%)	144 (96.6%)	(91.3%)

The association between gender and the presence of parainfluenza RNA:

According to the gender, 88 males and 61 females were included in the study. PIV types were detected in 8 males (9.1%) out of 88 while 5 females (8.2%) out of 61 were positive for HPIV RNA without significant difference between the two genders (*P* value =0.849) (Table. 3).

Table 3: The association between gender and the presence parainfluenza RNA among patients with respiratory symptoms Khartoum state, Sudan 2019:

HPIV		Gender		Total
		Males	Females	
Positive patients	No	8/88	5/61	13/149
	%	9.1%	8.2%	8.7%
Negative patients	No	80/88	56/61	136/149
	%	90.9%	91.8%	91.3%

(P value =0.849)

The association between the age groups and presence of Parainfluenza:

The patients were categorized into three age groups; among age group 0- less than 1 year 8/13 (61.5%) were positive for HPIV RNA, while among age group1-less than 2 years 4/13(30.8%) were positive for HPIV RNA, and among age group 4-5 years HPIV RNA

was detected in one sample (7.7%) (Table 4).

Table 4.: The association between age groups and the presence Parainfluenza RNA among patients with respiratory symptoms Khartoum state, Sudan 2019:

Age group	Frequency	Percent
0-1 year	8	61.5
1-2 year	4	30.8
4-5 year	1	7.7
Total	13	100.0

Correlation between clinical syndromes and the types of parainfluenza detected:

Among patients with bronchiolitis parainfluenza -3 was detected in 4 samples while neither HPIV -1,2 nor 4 RNA were detected. Among patients with pneumonia HPIV -3 was detected in 4 samples while HPIV -4 RNA were detected in 2 samples, in addition both HPIV -3 and HPIV -4 RNA were detected in one sample. For patients with bronchopneumonia; parainfluenza 4 was detected in 2 samples however HPIV -1,2 and 3 were not detected among patients with bronchopneumonia; without significant difference between clinical syndrome and parainfluenza serotypes (*P* value =0.115) (Table 5).

Table 5: Correlation between clinical syndromes and the types of Parainfluenza detected among patients with respiratory symptoms Khartoum state, Sudan 2019:

Diagnosis	Typing			Total
	para3	para4	para3+para4	
Bronchiolitis	4	0	0	4
Pneumonia	4	2	1	7
Bronchopneumonia	0	2	0	2
Total	8	4	1	13

***P*-value 0.115**

Isolation of parainfluenza virus using embryonated eggs:

Among parainfluenza RNA positive samples (n=13); no virus was isolated using embryonated eggs inoculation.

DISCUSSION

Human parainfluenza virus (HPIV) is known to cause acute respiratory infections (ARI), including lower respiratory tract infections, which are leading causes of morbidity and mortality in infants and young children worldwide [2]. Many reports from different countries have described the frequency of Parainfluenza virus and its subtypes, such as those reported by Indumathi et al., Villaran et al., and Pan et al. among patients with influenza-like illness in India, South America, and China, respectively [2, 39, 40]. However, no previous studies have been carried out to detect Parainfluenza viruses in Sudan.

The current study is the first to detect Parainfluenza viruses among respiratory patients in Sudan. In this study, Parainfluenza virus RNA was detected in 8.7% of patients with clinical respiratory syndromes (bronchiolitis, pneumonia, and bronchopneumonia). In 2013, Indumathi et al. in India reported that 11.2% were positive for Human parainfluenza virus using RT-PCR [2].

In addition, Villaran et al. in 2013 reported the frequency of Parainfluenza viruses in different regions in Central and South America, showing that 3.2% of patients with influenza-like illness were positive for HPIV. Most infected participants were male and under 5 years of age [39].

Moreover, Xiao et al. using Nested-PCR reported that 19.58% were positive for parainfluenza viruses in hospitalized children with Acute Lower Respiratory Infections in China [41].

In the current study, Human parainfluenza virus subtypes 3 and 4 were detected by RT-PCR. Parainfluenza virus type 3 was the most frequently (5.4%) detected subtype, followed by parainfluenza type 4 (3.4%), while neither human parainfluenza virus

type 1 nor human parainfluenza type 2 was detected. Furthermore, males were more frequently infected, and the infection was most common among patients under one year of age. Similarly, human parainfluenza virus type 3 was reported as the most frequently detected subtype in China by Indumathi et al., who also found that the other subtypes were not detected [2]. Additionally, parainfluenza virus subtypes were detected in 0.81%, 0.73%, 4.48%, and 0.57% for HPIV-1 to HPIV-4, respectively, as reported by Pan et al. in China. The highest yield rate of HPIV infection occurred in children under 5 years old, followed by patients over 60 years [40].

On the other hand, Sahar Essa et al. in Kuwait found that the most frequently isolated HPIV was HPIV-3 (71.8%), followed by HPIV-1 (23.1%). The least frequently isolated HPIV type was HPIV-4 (5.1%), and HPIV-2 was not detected. Infants ≤5 months of age were the most affected age group [6]. The study showed that HPIV-3 was most frequently detected among patients with bronchiolitis and pneumonia, while HPIV-4 was detected in patients with bronchopneumonia and pneumonia but with insignificant association. It is worth mentioning that HPIV-4 was found to have caused a localized outbreak of pneumonia and bronchiolitis, as reported by Xiao et al. in China [41].

No virus was isolated using embryonated egg inoculation. Eggs are a very poor growth medium for HPIV. It is only after many repeated passages and much presumed antigenic change that HPIV can be detected in embryonated eggs. Only three isolates of HPIV-2 have ever been reported to have undergone primary isolation in eggs [1].

With the application of molecular techniques, the detection of human parainfluenza viruses RNA and the subtypes among patients with flu-like illness and other related respiratory tract clinical manifestations has become faster and more reliable, aiding diagnosis and treatment, as well as providing information that directly influences the control measures for respiratory infection and disease progression. At the detection level, PCR remains the gold standard for confirmation of positive or negative results, especially for hard-to-diagnose infections such as HPIV.

The results obtained in our study should call for wider surveillance at the national level in order to fully unfold the true status and epidemiology of Human parainfluenza viruses and the other causative agents associated with respiratory syndromes in Sudan.

Finally, the present study represents the first to document the existence and incidence of HPIV and its types in Sudan through the detection of HPIV RNA in respiratory samples. Moreover, the HPIV detection using RT-PCR was established in the country. Generally, these findings are useful for future studies since there is no available information about HPIV infection in Sudan.

CONCLUSION

This study represents the first investigation into the prevalence of Human parainfluenza viruses (HPIV) among respiratory patients in Sudan. The findings reveal that 8.7% of patients with clinical respiratory syndromes, such as bronchiolitis, pneumonia, and bronchopneumonia, were positive for HPIV RNA. This is comparable to the prevalence rates reported in other regions, such as India (11.2%) and China (19.58%).

The study identified HPIV subtypes 3 and 4 as the most prevalent in Sudan, with HPIV-3 being the most frequently detected subtype at 5.4%, followed by HPIV-4 at 3.4%. This aligns with the global trend where HPIV-3 is often the most common subtype detected. Notably, HPIV-1 and HPIV-2 were not detected in this study, mirroring findings in other regions like India.

The demographic analysis indicated a higher infection rate in males and predominantly in children under one year of age. This is consistent with global data

showing that young children are the most vulnerable to HPIV infections.

The study also highlights the limitations of using embryonated eggs for virus isolation, as no virus was isolated using this method. The use of molecular techniques, particularly RT-PCR, proved to be more effective for detecting HPIV RNA and its subtypes. These molecular methods are crucial for accurate diagnosis, treatment, and the implementation of control measures for respiratory infections.

Finally, the present study represents the first to document the existence and incidence of HPIV and its types in Sudan through the detection of HPIV RNA in respiratory samples. Moreover, the establishment of HPIV detection using RT-PCR in the country is a significant advancement. These findings are useful for future studies, providing a foundational understanding of HPIV infection in Sudan, where no prior information was available.

In conclusion, the findings of this study underscore the need for extensive national surveillance to better understand the epidemiology of HPIV and other respiratory pathogens in Sudan. Such surveillance efforts will be instrumental in shaping public health strategies and mitigating the impact of respiratory infections on the population.

Declaration by Authors

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