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Investigation of influenza A of pandemic potential and MERS-Coronavirus in humans in Cameroon

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Abstract

Objective According to the World Health Organization, surveillance for respiratory viruses with pandemic potential should be included in routine surveillance to be on alert for zoonotic transmission. This study reports on data from the surveillance of influenza A/H5, influenza A/H7 and MERS-Coronavirus in Cameroon.

Results A total of 855 participants were enrolled. Of these, 11.7% were positive for influenza A and none were positive for influenza A/H5, A/H7 and MERS-Coronavirus. Most participants (77.1%) were enrolled within 5 days of illness onset and the younger population under 2 years of age (31.4%) was the most represented. In terms of clinical manifestations, the majority had flu-like symptoms including fever, cough, rhinorrhoea, asthenia, shortness of breath, noisy breathing and headache. These results are important to fill the knowledge gap on the epidemiology of influenza A/H5, A/H7 and MERS-Coronavirus in humans, for which information is lacking in several countries.

Keywords Surveillance, Influenza A/H5, Influenza A/H7, MERS-coronavirus, Severe acute respiratory infection, Human

Introduction

Influenza virus is an enveloped virus with a segmented negative-sense RNA genome belonging to the genus Orthomyxovirus [1]. This virus has four types that have been identified as causing disease in humans and animals [2]. Influenza A and B affect humans and cause seasonal epidemics in all countries, with the potential for influenza A to cause a pandemic; influenza C causes sporadic infections in humans, while influenza D causes infections in animals [1–3]. Influenza A is further sub-divided into subtypes based on antigenic variation in the surface haemagglutinin and neuraminidase proteins. There are now up to 18 subtypes of haemagglutinin and 11 subtypes of neuraminidase [2]. H1 and H3 are the most common subtypes found in seasonal influenza infections caused by A(H3N2) and A(H1N1)pdm09 viruses [2]. Meanwhile,

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H5, H7 and H9 have been identified as responsible for influenza outbreaks in animal species (wild birds, poultry, pigs, horses), causing enormous losses in the animal industry, with the ability to spill over and cause severe infections in humans [4]. Previous reports have shown human infections caused by the influenza viruses of subtypes H5, H7 and H9, particularly in North America, Asia and Africa, with mortality rates exceeding 30% [5–7]. H5 and H7 are considered as influenza viruses with pandemic potential because these viruses are antigenically novel to humans, lack pre-existing immunity and can adapt to cause sustained human-to-human transmission [5]. In addition, H5 and H7 are the only subtypes with the potential to mutate from low pathogenic avian influenza virus (LPAIV) to high pathogenic avian influenza virus (HPAIV) [8, 9].

Middle East respiratory syndrome-Coronavirus (MERS-CoV) was first identified in June 2012 in a 60-year-old man living in Saudi Arabia, who presented with acute pneumonia and later died of respiratory distress and renal failure [10]. Since 2012, 27 countries have reported cases of MERS-CoV infection, the majority of which were associated with contact with infected dromedary camels, resulting in a total of 858 deaths with a case fatality rate of 35% [11]. MERS-CoV is a single-stranded positive-sense RNA genome containing 10 predicted open reading frames (ORFs) [10]. Given that Saudi Arabia is a hotspot for MERS-CoV infection and hosts a large population participating in religious pilgrimages, this increases the pandemic potential for MERS-CoV [12].

Cameroon has experienced several outbreaks of avian influenza caused by HPAI of strains H5N8 (2006) and H5N1 (2016–2017) with mortality rates ranging from 8 to 96% [13, 14]. However, no human infections have been identified, although serological analysis has shown evidence of previous exposure and seroconversion in a minority of close human contacts [13, 15]. Meanwhile, no infections due to H7 and MERS-CoV have yet been identified in Cameroon.

According to the WHO guidelines, the surveillance of respiratory viruses with pandemic potential should be included in routine surveillance for the monitoring of these viruses. This study reports data from surveillance for influezna A/H5, A/H7 and MERS-CoV among people hospitalized with severe acute respiratory infections in Cameroon.

Methods

Study setting

This is a descriptive study performed from 2017 to 2022 at the Centre Pasteur Cameroon, the National Influenza Centre in Cameroon. Specimens were collected as part of surveillance of influenza viruses from persons with severe acute respiratory infections (SARI) from sentinel sites located in six regions of Cameroon (Centre, North, West, Littoral, South West and North West). SARI was defined as patients presenting with history of fever (or fever) and cough within 10 days of onset and requiring hospitalization. We employed stratified random sampling techniques to select specimens for the diagnosis of influenza A of pandemic potential (H5 and H7) and MERS-Coronavirus, ensuring a representative sample.

The samples were first stratified by year of enrolment. Then for each stratum, the sample size was set at 10% of the total samples collected for that year, corresponding to a sampling interval of 10. Specimens were organized in ascending order based on the date of enrolment, and every n^{th} sample was selected until the desired sample size was reached.

Specimen consisted of nasopharyngeal swab collected into 2mL cryovials containing viral transport medium.

Laboratory procedure

The QIAamp Viral RNA Mini Kit (Qiagen, Hilden, Germany) was used to extract RNA from 140µL of clinical samples into a final volume of 60µL elution buffer according to the manufacturer's instructions. After nucleic acid extraction, all samples were tested for the selected viruses using real-time PCR assays.

Extracts were tested for the presence of influenza A using the Centres for Disease Control and Prevention (CDC) influenza A typing assay in an ABI Prism 7500 thermocycler (Applied Biosystems, Foster City, California, USA). Positive samples were then subtyped using the CDC H5 and H7 subtyping kits. All PCR reactions for influenza virus and detection were performed using a SuperScript™ III Platinum one-step quantitative reverse transcription-polymerase chain reaction (RT-PCR) System (ThermoFisher Scientific, Massachusetts, USA). The reaction mixture was composed as follows: 12.5µL of 2X PCR Master Mix, 0.5µL of Reverse Transcriptase/Taq polymerase, 0.5µL of ROX (carboxy-X-rhodamine), 2µL of 10µM forward and reverse primers, 2µL of 2.5µM probe, and 5µL RNA extract. The following cycling conditions were used: one cycle of 30 min at 50 °C, followed by 95 °C for 2 min, and 45 cycles of amplification at 95 °C for 15 s and 55 °C for 30 s. Samples were considered positive for influenza A, H5 and H7 if the threshold cycles (Ct) were less than 37.

The MERS-CoV detection procedure targets two regions within the genome: the upper region of gene E is used for an initial screening while the Open Reading Frame (ORF) 1a is used to confirm the presence of the virus.

The following sets of primers and probes were used for amplification of the upper region of the E gene (upE sens: GCAACGCGCGATTCAGTT, upE antisens: GCCTCTA CACGGGACCCATA, upE probe: FAM-CTCTTCACA

TAATCGCCCCGAGCTCG-TAMRA) and ORF1a gene (EMC-Orf1a sens: CCACTACTCCCATTTCGTCAG, EMC-Orf1a antisens: CAGTATGTGTAGTGCGCATA TAAGCA, EMC-Orf1a probe: FAM-TTGCAAATTGG CTTGCCCCCACT-TAMRA). The reaction mixture for a total volume of 20uL was composed as follows: 3.6uL DNase/RNase free water, 0.4uL of 50mM MgSO4, 12.5uL of 2X Master Mix, 1uL each of sens and antisens primers, 0.5uL probe and 1uL enzyme (SSIII reverse transcriptase/Taq Polymerase). To the mix preparation, 5uL of each sample including negative and positive controls were added to the corresponding well. The thermal cycler was set as follows: one cycle of 20 min at 55 °C, one cycle of 3 min at 94 °C, 45 cycles of two-step amplification at 94 °C for 15 s and 58 °C for 30 s. All laboratory analyses were performed at the Virology Department of the CPC.

Additionally, all patients were screened for influenza B viruses, respiratory syncytial virus, and SARS-CoV-2 to confirm negative results for major circulating viral pathogens. SuperScript™ III Platinum one-step quantitative reverse transcription-polymerase chain reaction (RT-PCR) System (ThermoFisher Scientific, Massachusetts, USA) was utilized for screening of all three pathogens.

Testing for influenza B was conducted using the CDC influenza B typing assay in an ABI Prism 7500 thermocycler (Applied Biosystems, Foster City, California, USA). The reaction mixture and cycling conditions were similar to those used for the detection of influenza A, with specimens considered positive if the threshold cycle was below 37.

Specimens were tested for SARS-CoV-2 using the DaAn Gene molecular assay (DaAn Gene, Guangzhou, Guangdong Province, China), which targets the N and ORF1ab genes. The master mix consisted of 17 µL of PCR reaction mix, 3 µL of enzyme, and 5 µL of the extracted RNA, resulting in a final volume of 25 µL. RT-PCR amplification was performed using a QuantStudio 7 Flex (Applied Biosystems). Thermal cycling conditions for cDNA synthesis included 15 min at 50 °C and 15 min at 95 °C, followed by 45 amplification cycles at 94 °C for 15 s and 55 °C for 45 s. Results were considered positive if amplification of the N and ORF1ab genes yielded a threshold (Ct) of less than 37.

Specimens were tested for HRSV using the CDC protocol which targets the N gene. With a final volume of 25 ul, the master mix included 1 ul of the enzyme, 0.5 ul forward primer, 0.5 ul reverse primer, 0.5 ul probe, 12.5 ul of the reaction mix, 5 ul nuclease-free water, and 5 ul of the extracted RNA. Applied Biosystems QuantStudio 7 Flex was used for RT-PCR amplification. For cDNA synthesis, the thermal cycling settings were 10 min at 45 °C, 10 min of enzyme activation at 95 °C, 45 amplification cycles at 95 °C for 15 s, followed by 1 min at 55 °C. If the threshold (Ct) for N gene amplification was less than 37, the results were deemed positive.

Data analysis

Statistical analysis was performed using the Statistical Package for Social Sciences (SPSS) software version 22.0, while figures were generated using Microsoft 365 Excel software (Microsoft, Washington, DC, USA).

Ethical statement

Approval to use influenza surveillance data for research was obtained from the University of Douala Institutional Ethics Committee for Research on Human Health (N° 3971/CEI-Udo/07/2023/M) and the Cameroon National Ethics Committee (Reference N°: 2016/08/798/CE/CNERSH).

Results

Description of study participants

A total of 855 participants were enrolled from 2017 to 2022, with the years 2021 being the least represented. The majority originated from the Centre region (65.7%), followed by the West region (18.2%). Most participants (77.1%) were enrolled within 5 days of illness onset. The younger population aged 0-<2 (31.4%) and 2–4 (17.4) years were the most represented. The sex distribution was almost similar, but slightly in favour of males (Table 1).

Clinical characteristics of study participants

All participants in this study were febrile and had severe infections with varying symptoms. Figure 1 is a summary of the symptoms of the study participants. Most participants had cough (91.1%), rhinorrhoea (84.7%) and asthenia (80.1%). Other symptoms, although less common, included shortness of breath, noisy breathing, headache, and vomiting. The least common symptoms among the participants were conjunctivitis (13.1%), ear pain (8/8%) and skin rash (5.7%).

Results of screening of influenza A, A/H5, A/H7 and Mers-Coronavirus

A total of 100 samples tested positive for seasonal influenza A virus accounting for a positivity rate of 11.7%. Of these, none were positive for influenza A/H5 and A/H7. All samples tested were also negative for MERS-Coronavirus (Table 2).

Discussion

This study reports on a six-year investigation of influenza viruses with pandemic potential (H5 and H7) and MERS-Coronavirus in persons with severe respiratory infections in Cameroon. A random selection of specimen ensured a representative sample; however,, no cases of the target viruses were detected with the use of molecular assays.

Table 1 Socio-demographic characteristics of the study population

Characteristic	Sub-categories	Frequency	Percentage (%)
Year	2017	161	19.3
	2018	166	19.8
	2019	131	15.7
	2020	145	17.3
	2021	66	7.9
	2022	168	20.9
Region	Centre	550	65.7
	North	24	2.9
	West	152	18.2
	Littoral	37	4.4
	South West	13	1.6
	North West	61	7.3
Age	0-<2	263	31.4
	2–4	146	17.4
	5–14	84	10.0
	15–49	93	11.1
	Above 50	56	6.7
	Unknown	195	23.3
Sex	Male	327	39.1
	Female	314	37.5
	Unknown	196	23.4
Illness duration	0–5 days	659	77.1
	6–10 days	78	9.1
	Unknown	118	13.8
TOTAL		**855**	**100**

Table 2 Respiratory virus detection rate in the study population

Virus type	Tested *N*	Positive *n* (%)
Influenza A	855	100 (11.7)
Influenza A/H5	855	0
Influenza A/H7	855	0
MERS-Coronavirus	855	0

The choice of PCR over serology was made due to its higher sensitivity and specificity for detecting viral RNA, particularly in low-prevalence settings. This approach, while effective, may result in missed infections if viral loads are low, thus impacting the interpretation of our findings. Nonetheless, this study establishes a baseline for future surveillance efforts, highlighting the current low risk of transmission to humans.

These results are consistent with the epidemiology of avian influenza in Cameroon, as there was no recorded outbreak during the study period: the last outbreak spanned from May 2016 to March 2017 [14]. The outbreak was caused by the HPAI H5N1 clade 2.3.2.1c and resulted in more than 40,000 bird deaths [13, 14] and seroconversion in a few human cases [15]. Because of the potential for HPAI to cross species barriers, it is important to monitor the virus regularly in animals and humans. This is supported by a recent report of a severe case of H5N1 infection in a 9-year-old child in Ecuador, Latin America, following contact with dead backyard

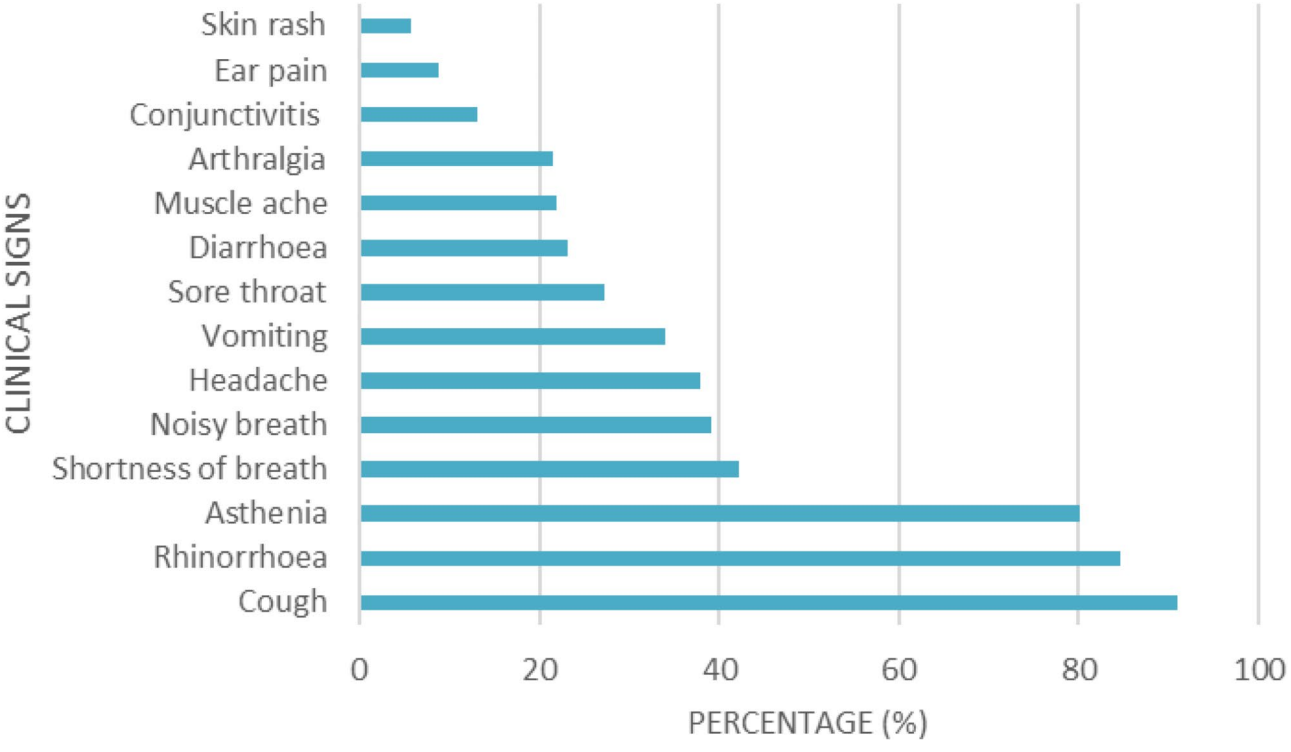

Fig. 1 Clinical characteristics of the study population

poultry [16]. Similarly, the very first human outbreak of H5N1 in 1997, in which 6 of 18 people died, would have gone undetected without investigation and surveillance of the human and poultry populations [17]. Other human outbreaks and infections due to A/H5 and A/H7 have been detected through active surveillance [6, 18–22].

For MERS-Coronavirus, there is no evidence of circulation of this virus in Cameroon, but several studies conducted in Africa have shown the presence of the virus in livestock [23]. During the period of 2017–2022, human cases of MERS-Coronavirus were reported in the Middle East (Saudi Arabia, United Arab Emirates, Qatar, Oman, Lebanon), Asia (Republic of Korea, Malaysia) and Europe (United Kingdom). International travel and uncontrolled movement of livestock from one country to another increase the likelihood of spread of MERS-Coronavirus from infected animals or human cases. Surveillance of this virus is therefore essential to be on alert for any animal-to-human or human-to-human transmission. In addition, over 5,000 Cameroonians make the annual pilgrimage to Mecca, Saudi Arabia [24], which is a high-risk zone for MERS-Coronavirus.

Regarding the clinical manifestation of participants, the majority had flu-like symptoms including fever, cough, rhinorrhoea, asthenia, shortness of breath, noisy breathing and headache. Previous studies have also shown that avian influenza and MERS-Coronavirus infections can be asymptomatic or symptomatic with a range of severity from mild upper respiratory illness, to severe pneumonia, multiple organ failure and ultimately death [17, 25, 26]. Unfortunately, we did not have additional information to link other potential causes of infection in these individuals, such as their history of infection, exposure to animal reservoirs, knowledge of other similar cases in the close environment, and travel history. Additionally, all patients were screened for common viral pathogens, including influenza B viruses, respiratory syncytial virus, and SARS-coronavirus, to confirm negative results for major circulating pathogens.

The results of this study, although negative for all the viruses sought, are important in filling the knowledge gap on the epidemiology of influenza A/H5, A/H7 and MERS-Coronavirus in humans, for which information is lacking in several countries. As seasonal influenza A, which can cross species barrier and cause pandemics represented 11.7% of all severe acute respiratory infections, this study highlights the role of the influenza surveillance system in preparedness to future pandemic. Furthermore, this study highlights the need to search for other pathogens that could be responsible for severe respiratory infections in Cameroon, particularly for the 35–80% of patients who are negative for the most common viral and bacterial pathogens, including influenza, respiratory syncytial virus, rhinovirus, coronavirus, *Haemophilus influenzae* and *Klebsiella pneumoniae* [27–29].

Limitations

The absence of information on the potential exposures of participants to poultry and swine or information on travel history are identified gaps in the surveillance of these infections. These need to be addressed in order to be better prepared in case of future infection or outbreak due to the A/H5, A/H7, MERS-Coronavirus, or other respiratory viruses capable of causing pandemics. Future research should focus on expanding surveillance to include a broader range of pathogens and to gather comprehensive exposure histories for affected individuals.

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Author contributions
Conceptualization, R.N.; methodology, G.C.M., R.N. and M.N.R.; software, G.C.M; validation, R.P. and R.N.; formal analysis, G.C.M.; investigation, M.N.R., C.N.Y., D.T.T., H.L.M.N.; re-sources, R.N., U.T., C.N.B.; data curation, G.C.M. and L.L.M.E.; writing—original draft prepara-tion, G.C.M.; writing—review and editing, G.C.M., M.N.R., C.N.Y., L.L.M.E., C.N.B., D.T.T., H.L.M.N., U.T., R.P. and R.N.; supervision, C.N.B., R.P. and R.N.; funding acquisition, R.N. and U.T. All authors have read and agreed to the published version of the manuscript.

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Data availability
Data is provided within the manuscript or supplementary information files.

Declarations

Competing interests
The authors declare no competing interests.

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