

Molecular detection and prevalence of SARS-CoV-2 during the early outbreak in Southern Bangladesh

Eaftekhar Ahmed Rana¹, Nahida Sarwer Chowdhury², Md. Sirazul Islam², Jahan Ara², Syeda Shamima Nasrin², Pronesh Dutta², Sabiha Zarin Tasnim Bristi², Tanvir Ahmad Nizami², Prashanta Chakraborty² and Amam Zonaed Siddiki³

1. Department of Microbiology and Veterinary Public Health, Chattogram Veterinary and Animal Sciences University, Chattogram, Bangladesh; 2. COVID-19 Diagnostic Laboratory, Chattogram Veterinary and Animal Sciences University, Chattogram, Bangladesh; 3. Department of Pathology and Parasitology, Chattogram Veterinary and Animal Sciences University, Chattogram, Bangladesh.

Corresponding author: Md. Sirazul Islam, e-mail: sirazul@yahoo.com

Co-authors: EAR: eaftekhar787@gmail.com, NSC: nahidasarwer@gmail.com, JA: arajahan155@gmail.com, SSN: s.nasrin97@gmail.com, PD: proneshdutta92@gmail.com, SZTB: zarinsabiha@gmail.com, TAN: tanvir.vet@gmail.com, PC: pcarkgebcu@gmail.com, AZS: zsiddiki@gmail.com

Received: 22-08-2020, **Accepted:** 05-10-2020, **Published online:** 03-11-2020

doi: www.doi.org/10.14202/IJOH.2020.153-159 **How to cite this article:** Rana EA, Chowdhury NS, Islam MS, Ara J, Nasrin SS, Dutta P, Bristi SZT, Nizami TA, Chakraborty P, Siddiki AZ (2020) Molecular detection and prevalence of SARS-CoV-2 during the early outbreak in Southern Bangladesh, *Int. J. One Health*, 6(2): 153-159.

Abstract

Background and Aim: Coronavirus disease (COVID-19) has been announced as a life-threatening, highly transmissible infectious novel emerging disease worldwide. Rapid detection and epidemiological information are desperately needed to overcome the existing pandemic situation and alleviate national and international crises. Still, to date, there is no significant epidemiological study of COVID-19 available in Bangladesh, especially in the Chattogram division. However, the current study focuses on molecular detection, prevalence, and risk factors associated with COVID-19 in Southern Bangladesh.

Materials and Methods: Standard diagnostic protocols through real-time reverse transcriptase-polymerase chain reaction (RT-qPCR) were performed for molecular confirmation of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Different patient demographics were analyzed for exploring the relationship of four factors – region, sex, age, and symptoms with the accumulated number of COVID-19 cases in the Southern Bangladesh during the period of May 13, 2020, to June 12, 2020.

Results: A total of 2954 samples were tested where the cumulative prevalence of circulating SARS-CoV-2 was 29.76% (n=879; 95% CI: 28.11-31.44) in the selected study region. Among the risk factors, the present study revealed that flatland people (35.62%, 95% CI 33.61-37.67, OR=3.13) were more vulnerable to getting infected by SARS-CoV-2 than the people living in hill tracts (13.04%, 95% CI 10.73-15.63). People older than 50 years (34.68%, 95% CI 30.38-39.18) were designated the highest risk than other different age groups. A higher number of COVID-19 cases were confirmed in patients (36.0%, 95% CI 33.77-38.29, OR=1.76) with typical symptoms, but interestingly a significant number of asymptomatic carriers (20.39%, 95% CI 18.13-22.80) also positive for SARS-CoV-2.

Conclusion: To the best of our knowledge, this is the first epidemiological report in the Southern Bangladesh for COVID-19. The study's findings will contribute to health professionals and the policy-makers to take preventive measures against the next outbreak emergency for Bangladesh.

Keywords: asymptomatic carrier, coronavirus disease 2019, prevalence, risk factors, severe acute respiratory syndrome coronavirus 2.

Introduction

Within the past two decades, two notable coronaviruses – Severe acute respiratory syndrome coronavirus (SARS-CoV) [1] and Middle East respiratory syndrome coronavirus (MERS-CoV) [2] have emerged and subsequently caused two fatal diseases, namely, SARS and MERS, respectively [3,4]. In

December, 2019, a novel coronavirus introduced as SARS-CoV-2 [5] was evolved at Wuhan in the Hubei Province of China, causing coronavirus disease 2019 (COVID-19) [6,7]. Besides other significant coronavirus properties, fast transmissibility rate, and mortality due to the comorbidity made SARS-CoV-2 more dangerous than previously evolved other coronaviruses namely SARS-CoV and MERS-CoV [8-10]. Before the declaration of the COVID-19 outbreak as a pandemic by the WHO, Bangladesh confirmed its first three COVID-19 patients cases in March 8, 2020 [11]. However, Chattogram (previously known as Chittagong) is the largest division of Bangladesh, located in its southern part where the largest port is located beside the city [12]. Due to geographical

Copyright: Rana, et al. This article is an open access article distributed under the terms of the Creative Commons Attribution 4.0 International License (<http://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, distribution, and reproduction in any medium, provided you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made. The Creative Commons Public Domain Dedication waiver (<http://creativecommons.org/publicdomain/zero/1.0/>) applies to the data made available in this article, unless otherwise stated.

position and socio-economic context, it is the main export-import zone of this country [13]. For economic and trading purposes, multiple classes of people from different countries come close together and are involved with unrestricted movements. For these reasons, this location is considered as a highly infectious zone for contact transmission of SARS-CoV-2. In Chattogram, the first three cases of COVID-19 were detected on March 23, 2020 [14]. According to the WHO's report, July 2020, the Chattogram division had the 2nd highest COVID-19 attack rate in Bangladesh with linear growth of confirmed cases in all 11 districts of the Chattogram division.

Geographically, the landscape of the eastern region of Chattogram division is a hilly and remote area widely known as Chattogram hill tract (CHT) where a significant number of ethnic groups dwell together [15,16]. Simultaneously, the other regions of this division are flatland and more densely populated than the CHT area [17]. Moreover, the flatland region is highly connected with the other parts of the country and thrives economically with industrialization and international business [18]. On the other hand, the CHT area is less populated with ethnic communities and mostly dependent on subsistence agriculture and livestock [17,19]. However, both of these regions hold unique demographic, socio-economic, and community characteristics. Moreover, the variations of these characteristics significantly impact the epidemiology of SARS CoV-2 by affecting the community people [20,21].

Considering all the facts, including the geological position, demography, socio-economic status, and community characteristics of the Chattogram division, it is timely demand to find out the infectious pattern and different factors linked with the COVID-19 outbreak. This study aims to confirm the COVID-19 cases from suspected individuals and perform an epidemiological analysis to investigate the specific factors responsible for aggravating the COVID-19 cases in Chattogram, Bangladesh.

Materials and Methods

Ethical approval and informed consent

Before the sample collection, verbal permission from every suspected individual was taken, and subsequently two swab samples were collected with minimum discomfort of patients for confirmation of COVID-19. Finally, consent from the Research and Extension director of Chattogram Veterinary and Animal Sciences University (CVASU) was taken to conduct the current study.

Study area

The current study was conducted in the southern part of Bangladesh. Chattogram is the largest division in southern Bangladesh comprising of eleven districts [22]. However, seven districts of Chattogram were included in the present study. Khagrachari, Rangamati, and Bandarban districts are recognized as CHT, and the remaining four districts, including Feni,

Noakhali, Laxmipur, and Chattogram districts are known as flatland according to geographical position (Figure-1) [22].

Study population

In total, 2954 suspected individuals with relevant clinical sign symptoms such as fever, respiratory distress, cough, sneezing, headache, and loss of taste or smell reported to local and regional health complexes from May 13, 2020 to June 12, 2020 (1 month) were included as the target population. Any asymptomatic persons having contact history with previously confirmed COVID-19 cases were also sampled in current study.

Sample collection

The authorities of reported health complexes collected oropharyngeal and nasal swabs from the suspected individuals by following the WHO guidelines [23], where two separate swab samples were preserved in normal saline containing collection tubes. Immediately after collection of samples, proper cold chain was maintained and sent to the COVID-19 diagnostic laboratory of CVASU, Chattogram. Individual patient data were also sent to the laboratory for further details epidemiological analysis.

Molecular diagnosis

RNA extraction and RT-qPCR

The presence of SARS-CoV-2 in respiratory specimens was detected by the real-time RT-PCR method. Viral RNA was extracted from the patient's derived samples using SanSure Biotech Sample Release Reagent (SanSure Biotech) (Ref. No – S1014E), according to the manufacturer's instruction. PCR qualitative detection of the N gene (ROX channel) and ORF1ab region (FAM channel) of SARS-CoV-2 was detected using Novel Coronavirus (2019-nCoV) Nucleic Acid Diagnostic Kit (PCR-Fluorescence Probing) (Ref. No- S3102E) from SanSure biotech. Human RNA gene (CY5 channel) was used as an internal control to monitor PCR inhibition. Real-time RT-PCR was performed on an Applied BioSystems 7500 real-time PCR system with the Step One software version 2.3 for analysis.

Statistical analysis

All field derived data, including geographical location, sex, age, and symptoms were recorded into a Microsoft Excel 2010 spreadsheet and sorted for a better presentation of outcomes. Descriptive and analytical analyses were performed in STATA®13.0 software. Chi-square test was performed to find out the association of the binary result of SARS-CoV-2 with the patient's factors. Univariate and multivariate logistic regression analyses were performed to identify possible patients' risk factors.

Results

The overall prevalence of circulating SARS-CoV-2 positive cases in the Chattogram division was

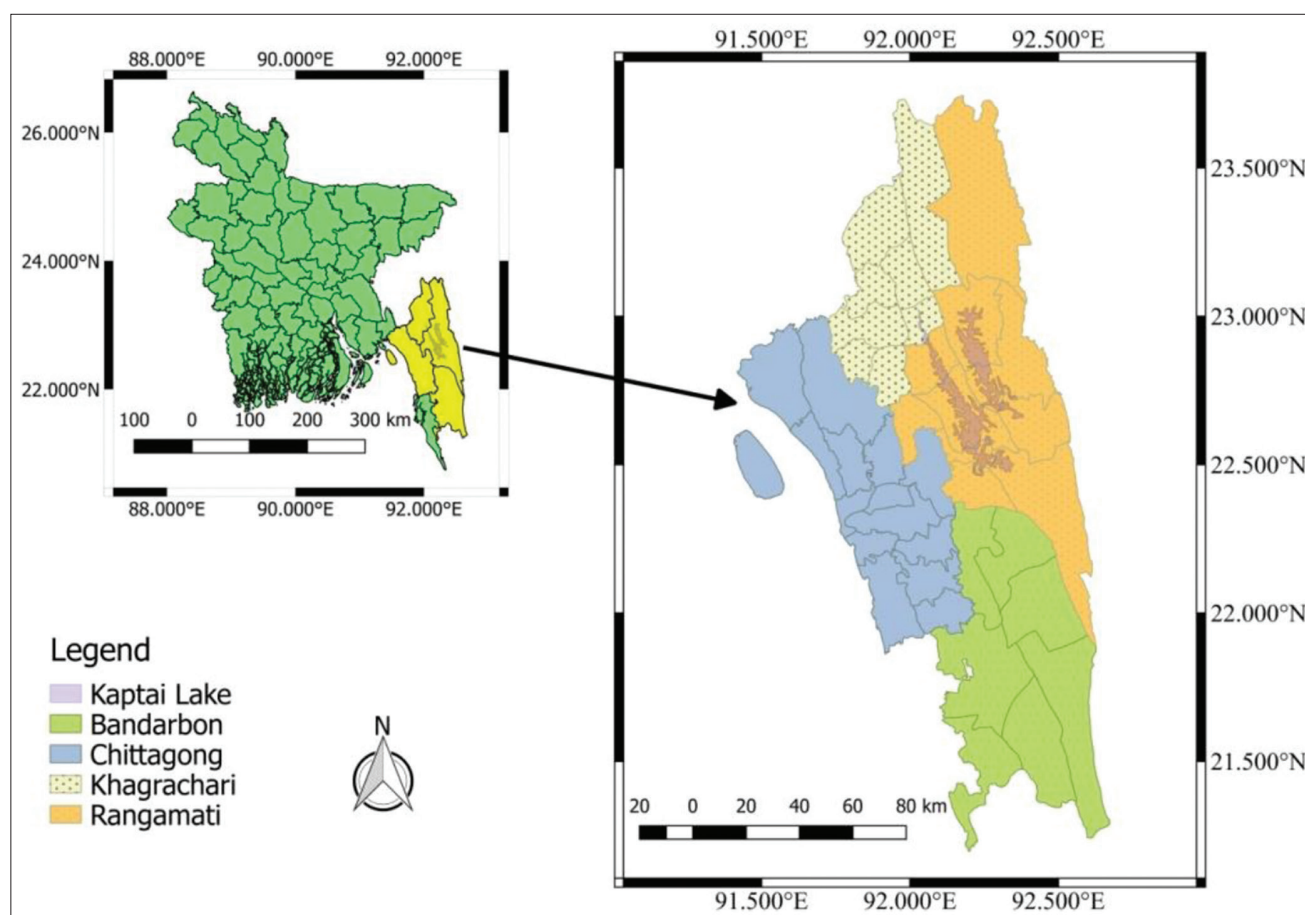


Figure-1: Geographical location of the study areas of Southern Bangladesh.

29.76% ($n=879$; 95% CI: 28.11-31.44). The prevalence was found 35.62% (95% CI 33.61-37.67) in flatland and 13.04% (95% CI 10.73-15.63) in the hill tract region. Besides the geographical location, the prevalence was 30.55% (95% CI 28.62-32.54) in males and 27.55% (95% CI 24.45-30.82) in females. Among the different age groups, old and adolescent groups had 34.68% (95% CI 30.38-39.18) and 33.10% (95% CI 25.52-41.39) infection rate, respectively, which were higher than other age groups (Table-1). However, 36% (95% CI 33.77-38.29) of patients expressed clinical signs and symptoms of SARS-CoV-2, whereas 20.39% (95% CI 18.13-22.80) positive individuals had no symptoms at all (Table-1).

Comparative prevalence of COVID-19 in accordance with the geological position

Within the flatland and hill tract region, the patient demographic showed distinct results for positive cases of COVID-19. The prevalence of symptomatic and asymptomatic positive patients was reported 39.58% and 27.18% in flatland wherein the hill tract region; it was found 17.25% and 10.56%, respectively. In the sex group category, this prevalence was recorded 35.97% in flatland and 14.23% in the hill tract region for the male group, which was slightly higher than the female group (34.59% in flatland and 10.18% in hill tract) (Figure-2).

Risk factors associated with the carriage of SARS-CoV-2 and suspected patient

The univariate analysis identified four potential risk factors ($p \leq 0.20$) associated with the presence of SARS-CoV-2. In univariate logistic regression, the flatland population was at almost 3.7 times more risk than the hill tract population of having COVID-19 infection. Secondly, males ($OR=1.16$) had an increased positive case than females. In the case of age, older people ($OR=1.8$) were at higher risk groups, followed by adolescents, young, middle-aged, and children at the lowest risk. Finally, for patients with symptoms, the odds of developing the disease was 2.2 times greater than the asymptomatic patients (Table-1).

In the subsequent multivariate logistic regression model, two different characteristics were found to be positively associated with the presence of SARS-CoV-2. Among the significant variables, people living in flatland found 3 ($OR=3.13$) times more risk of having SARS-CoV-2 infection than the hill tract population ($p < 0.001$). The suspected patient with clinical signs and symptoms showed a more favorable SARS-CoV-2 infection rate than the asymptomatic patient ($OR=1.76$, $p < 0.001$). Besides this, the suspected males of the Chattogram division were 1.13 times more at risk of being infected with SARS-CoV-2 infection than the females. At the age group, the adolescent group was 1.69 times higher at risk of

developing this viral disease followed by the old aged group (OR=1.5), the young age group (OR=1.31), and the middle age group (OR=1.17) than the child age group (Table-2).

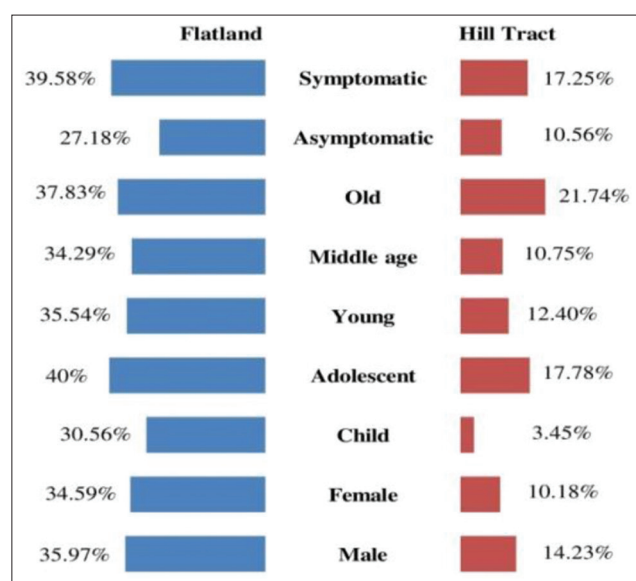


Figure-2: Comparative prevalence of COVID-19 in association with different demography of patients within Flatlands and Hill tracts region of Chattogram division.

Frequency of samples tested and the rate of COVID-19 positive cases within the study period

An uprising pattern of suspected sample numbers and positive cases of SARS-CoV-2 was disclosed in the study's analysis. Within the study time, 532 samples were tested in the 1st week, and subsequently 631, 815, and 976 samples were tested second, third, and 4th week, respectively (Figure-3). In last week, positive cases increased (29.81%) more than double compared with the 1st week (25.19%) of the study. In the research finding, the highest infection rate was observed in the 3rd week (33.25%), followed by the 4th week (29.81%) and 2nd week (29%).

Discussion

The third global challenge SARS-CoV-2 is now in the form of a pandemic after the SARS and MERS outbreak [24]. Prediction of the epidemiological trend of the disease is crucial for the control and prevention of the epidemic. The current study reveals that the overall prevalence of SARS-CoV-2 was 29.76% in the Southern Bangladesh within the study period. This finding is consistent with a study where the prevalence was 33% reported in New York [25]. On the other hand, it is distinctly higher than another survey conducted in Indiana, USA, where the prevalence was

Table-1: Univariate logistic regression analysis of selected risk factors for the presence of SARS-CoV-2 in suspected patients in different regions of Chattogram division.

Variables	Co-variable	SARS-CoV-2		Percentages of positive cases	95% CI	OR (95% CI)	p-value (Chi-square)
		Negative case	Positive case				
Region	Hill tract	667	100	13.04	10.73-15.63	Ref	<0.001*
	Flatland	1408	779	35.62	33.61-37.67	3.7(2.94-4.63)	
Sex	Female	568	216	27.55	24.45-30.82	Ref	0.115
	Male	1507	663	30.55	28.62-32.54	1.16 (0.96-1.39)	
Age	Child (0-10 years)	78	23	22.77	15.02-32.18	Ref	0.046*
	Adolescent (11-19 years)	97	48	33.10	25.52-41.39	1.68 (0.94-3.0)	
	Young (20-35 years)	992	408	29.14	26.77-31.60	1.39 (0.86-2.25)	
	Middle age (36-50 years)	601	237	28.28	25.25-31.46	1.34 (0.82-2.18)	
	Old (50+ years)	307	163	34.68	30.38-39.18	1.8 (1.1-2.97)	
Symptoms	Absent	941	241	20.39	18.13-22.80	Ref	<0.001*
	Present	1134	638	36.0	33.77-38.29	2.2 (1.85-2.61)	

CI=Confidence interval, OR=Odds ratio, Ref=Reference, SARS-CoV-2=Severe acute respiratory syndrome coronavirus 2, *Statistically significant values

Table-2: Multivariate logistic regression model for assessing the risk factors associated with SARS-CoV-2 and suspected patient in different regions of Chattogram division.

Variables	Covariable	OR	95% CI	p-value
Region	Hill tract	Ref		
	Flatland	3.13	2.48-3.95	<0.001*
Sex	Female	Ref		
	Male	1.13	0.94-1.37	0.198
Age	Child (0-10 years)	Ref		
	Adolescent (11-19 years)	1.69	0.93-3.07	0.086
	Young (20-35 years)	1.31	0.80-2.15	0.276
	Middle age (36-50 years)	1.17	0.71-1.94	0.53
	Old (50+ years)	1.5	0.9-2.52	0.122
Symptoms	Absent	Ref		
	Present	1.76	1.47-2.1	<0.001*

OR=Odds ratio, CI=Confidence interval, Ref=Reference, *Statistically significant values

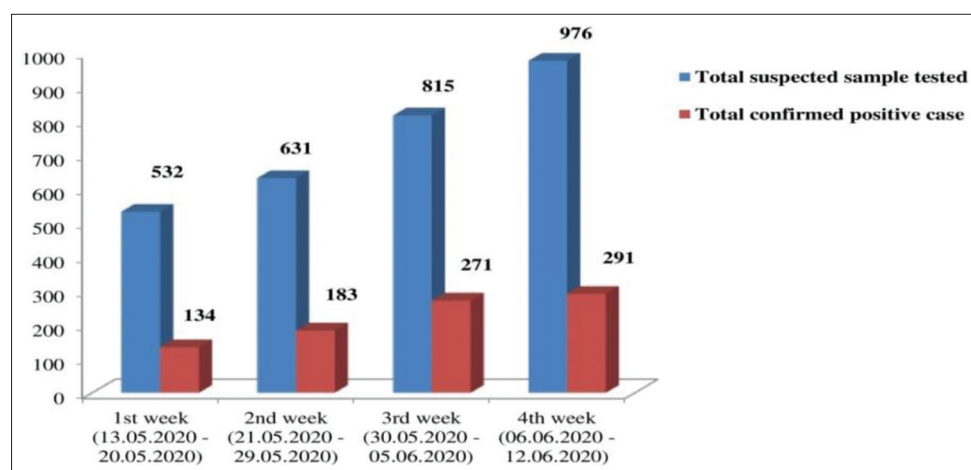


Figure-3: Cumulative number of suspected samples tested and confirmed COVID-19 cases (weekly) in the Chattogram division, Bangladesh.

reported at 1.74% [26]. This variation of infections' prevalence may be due to virus susceptibility toward the host body and different geographical and environmental factors, including land, weather, and movements of asymptomatic carrier patients.

The study also unveils the geographical variation of the SARS-CoV-2 infection rate within CHT and flatlands in the Chattogram division. The Chattogram division's flatlands had a 35.62% prevalence of SARS-CoV-2, which was 3 (OR=3.13) times higher than CHT. This distinction may result from livelihood, distinct cultures, and traditions of ethnic minorities in CHT, which differ from the way of the lifestyle of people living in flatlands [27]. The previous studies have shown that environmental, socio-economic, and demographic variables are positively associated with COVID-19 incidence [28]. Another study [29] has established a strong association between ethnicity and outcome of COVID-19, which is in harmony with the research findings. In terms of infectivity, the current analysis demonstrates that men are more vulnerable to COVID-19 than women. Other studies support a higher incidence in the male population: 56.3%, 58.1%, and 67% in New York and China, respectively [25,30,31]. The sex variations of infection rates might be due to differences in physiological parameters, including the expression of susceptible receptors for virus attachment, metabolic differences, and other habits different from females. The relevant findings of all Cleveland Clinics in Ohio and Florida narrated that males were at higher risk of being positive for COVID-19 [32], while most of the studies showed a higher prevalence in men, but some studies also indicated the approximately equal sex distributions [33,34]. Although the reasons for these differences are unknown, studies have revealed crucial information such as men are more likely to be current smokers and a higher proportion of comorbid conditions [35]. Sex and smoking concerning a higher expression of ACE2 (the receptor for SARS-CoV-2) [36] and the difference in sex hormones might be the causes of being vulnerable in males [37]. The age distribution of

patients from this study indicates that adolescents are as likely to be infected as adults. Also consistent with early reports [25,31], younger people are less likely to be affected, supported by several studies. All those research findings suggest that practical efforts should be made to protect and reduce transmission and symptom progression in vulnerable populations, including elderly and young children.

According to study findings, 20.39% of positive individuals reported asymptomatic COVID-19 patients who are close to the results of Jinan, China (23.41%) [38], and Daegu, South Korea (19.2%) [39]. Asymptomatic might have certain variations of the ACE2 gene, making them less susceptible to getting infected by the novel coronavirus's spike protein [40]. This study has some limitations, including a lack of demographic characteristics of patients treated intensively during the infection period to the recovery stage, and no accurate therapeutics guideline was recorded for recovered COVID-19 positive patients. Therefore, a long-term, well-designed epidemiological study will be required to better understand the pandemic nature of viral diseases like the COVID-19 outbreak.

Conclusion

The present study reveals that the overall prevalence of SARS-CoV-2 was 29.76%, where 36.0% suspected individuals showed clinical symptoms, and 20.39% did not express any signs for the COVID-19. In epidemiological analysis focused on people who live in flatland regions and aged more than 50 years were found to a higher risk for SARS-CoV-2 infection. However, the present study findings undoubtedly support physicians and legislators. For a precise understanding of transmission dynamics of circulating virus and better control measures against COVID-19 outbreak throughout the country, including Chattogram division.

Authors' Contributions

EAR was responsible for the planning, designing, and primary drafting of the manuscript. NSC,

MSI, JA, SSN, PD, SZTB, TAN, and PC contributed to sample preparation and molecular diagnosis (RT-PCR). NSC executed the data entry and sorting. PD performed statistical analyses. SSN and JA were responsible for the literature review. MSI interpreted and guided the manuscript preparation. AZS revised the final manuscript and gave critical corrections. All authors read and approved the final manuscript.

Acknowledgments

The authors are thankful to Prof. Dr. Goutam Buddha Das, Vice-chancellor, Chattogram Veterinary and Animal Sciences University, for the emergency establishment of the COVID-19 diagnostic laboratory during the pandemic situation. The authors acknowledge Bangladesh Institute of Tropical and Infectious Diseases for assisting samples and patient data collection from different regions of Chattogram division. In the present study, the laboratory diagnostic facility was supported by the Directorate General of Health Services, Bangladesh by providing RNA extraction reagent as well as RT-PCR kits and the Poultry Research and Training Center, CVASU for others logistic support.

Competing Interests

The authors declare that they have no competing interests.

Publisher's Note

Veterinary World (Publisher of International Journal of One Health) remains neutral with regard to jurisdictional claims in published map and institutional affiliation.

References

1. Peiris, J.S.M., Lai, S.T., Poon, L.L.M., Guan, Y., Yam, L.Y.C., Lim, W., Nicholls, J., Yee, W.K.S., Yan, W.W., Cheung, M.T. and Cheng, V.C.C. (2003) Coronavirus as a possible cause of severe acute respiratory syndrome. *Lancet*, 361(9366): 1319-1325.
2. Zaki, A.M., van Boheemen, S., Bestebroer, T.M., Osterhaus, A.D. and Fouchier, R.A. (2012) Isolation of a novel Coronavirus from a man with pneumonia in Saudi Arabia. *N. Engl. J. Med.*, 367(19): 1814-1820.
3. de Wit, E., van Doremalen, N., Falzarano, D. and Munster, V.J. (2016) SARS and MERS: Recent insights into emerging coronaviruses. *Nat. Rev. Microbiol.*, 14(8): 523-534.
4. Su, S., Wong, G., Shi, W., Liu, J., Lai, A.C., Zhou, J., Liu, W., Bi, Y. and Gao, G.F. (2016) Epidemiology, genetic recombination, and pathogenesis of Coronaviruses. *Trends Microbiol.*, 24(6): 490-502.
5. International Committee on Taxonomy of Viruses. (2020) Naming the Coronavirus Disease (COVID-19) and the Virus that Causes It. Available from: [https://www.who.int/emergencies/diseases/novel-coronavirus-2019/technical-guidance/naming-the-coronavirus-disease-\(covid-2019\)-and-the-virus-that-causes-it](https://www.who.int/emergencies/diseases/novel-coronavirus-2019/technical-guidance/naming-the-coronavirus-disease-(covid-2019)-and-the-virus-that-causes-it). Retrieved on 17-09-2020.
6. Zhu, N., Zhang, D., Wang, W., Li, X., Yang, B., Song, J., Zhao, X., Huang, B., Shi, W., Lu, R. and Niu, P. (2020) China novel Coronavirus investigating and research team. A novel Coronavirus from patients with pneumonia in China, 2019. *N. Engl. J. Med.*, 382(8): 727-733.
7. Perlman, S. (2020) Another decade, another coronavirus. *N. Engl. J. Med.*, 382(8): 760-762.
8. Guo, Y.R., Cao, Q.D., Hong, Z.S., Tan, Y.Y., Chen, S.D., Jin, H.J., Tan, K.S., Wang, D.Y. and Yan, Y. (2020) The origin, transmission and clinical therapies on Coronavirus disease 2019 (COVID-19) outbreak-an update on the status. *Mil. Med. Res.*, 7(1): 11.
9. Michalakis, K., Goulis, D.G., Vazaiou, A., Mintziori, G., Polymeris, A. and Abrahamian-Michalakis, A. (2013) Obesity in the ageing man. *Metabolism*, 62(10): 1341-1349.
10. Mehta, V., Goel, S., Kabarriti, R., Cole, D., Goldfinger, M., Acuna-Villaorduna, A., Pradhan, K., Thota, R., Reissman, S., Sparano, J.A. and Gartrell, B.A. (2020) Case fatality rate of cancer patients with COVID-19 in a New York hospital system. *Cancer Discov.*, 10(7): 935-941.
11. Saha, S., Malaker, R., Sajib, M.S.I., Hasanuzzaman, M., Rahman, H., Ahmed, Z.B., Islam, M.S., Islam, M., Hooda, Y., Ahlyong, V. and Vanaerschot, M. (2020) Complete genome sequence of a novel Coronavirus (SARS-CoV-2) isolate from Bangladesh. *Microbiol. Resour. Announc.*, 9(24): e00568-e00620.
12. Government of the People's Republic of Bangladesh. (2020) Population-and-Housing-Census-Bangladesh Bureau of Statistics-Government of the People's Republic of Bangladesh. Available from: <http://www.bbs.gov.bd/site/page/47856ad0-7e1c-4aab-bd78-892733bc06eb/population-and-housing-census>. Retrieved on 17-09-2020.
13. Sun, Z., Zhang, H., Yang, Y., Wan, H. and Wang, Y. (2020) Impacts of geographic factors and population density on the COVID-19 spreading under the lockdown policies of China. *Sci. Total Environ.*, 746: 141347.
14. World Health Organization. (2020) Coronavirus Disease (COVID-2019) Bangladesh Situation Report-06. World Health Organization, Bangladesh.
15. United Nations International Children's Emergency Fund. (2019) Many Tracts One Community-UNICEF'S Work in the Chittagong Hill Tracts, 2019. United Nations International Children's Emergency Fund, Bangladesh. Available from: <https://www.unicef.org/bangladesh/media/2601/file/cht%20report.pdf>. Retrieved on 17-09-2020.
16. United Nations Development Programme, Ministry of Chittagong Hills Tracts Affairs. (2020) Promotion of Development and Confidence Building in the Chittagong Hill Tracts Project. United Nations Development Programme, Bangladesh and Ministry of Chittagong Hills Tracts Affairs, Chittagong. Available from: http://www.eeas.europa.eu/archives/docs/top_stories/description_chittagong_en.pdf. Retrieved on 17-09-2020.
17. Asia-Pacific Forestry Sector Outlook Study. (2020) Country Report-Bangladesh. Available from: <http://www.fao.org/3/y0165e/y0165e02.html>. Retrieved on 17-09-2020.
18. Mia, M.A., Nasrin, S., Zhang, M. and Rasiah, R. (2015) Chittagong, Bangladesh. *Cities*, 48: 31-41.
19. Hossain, D.M. (2013) Socio-economic situation of the Indigenous people in the Chittagong Hill Tracts (CHT) of Bangladesh. *Middle East J. Bus.*, 8(2): 22-30.
20. Wadhera, R.K., Wadhera, P., Gaba, P., Figueroa, J.F., Maddox, K.E.J., Yeh, R.W. and Shen, C. (2020) Variation in COVID-19 hospitalizations and deaths across New York city boroughs. *J. Am. Med. Assoc.*, 323(21): 2192-2195.
21. Yancy, C.W. (2020) COVID-19 and African Americans. *J. Am. Med. Assoc.*, 323(19): 1891-1892.
22. List of Districts-Chattogram Division. (2020) Available from: <http://www.chittagongdiv.gov.bd/site/page/98079815-2144-11e7-8f57-286ed488c766/listofdistricts&upazila>. Retrieved on 17-09-2020.
23. World Health Organization. (2020) Laboratory Testing for Coronavirus Disease 2019 (COVID-19) in Suspected Human Cases. World Health Organization, Geneva. p1-7.
24. Mohammadzadeh, N., Shahriari, M., Shirmohammadlou, N. and Lohrasbi, V. (2020) A glance at the prevalence of Coronavirus disease 19 (COVID-19) in Iran: Strengths and weaknesses. *Infect. Control Hosp. Epidemiol.*, 2020: 1-3.

25. Rosenberg, E.S., Dufort, E.M., Blog, D.S., Hall, E.W., Hoefler, D., Backenson, B.P., Muse, A.T., Kirkwood, J.N., George, K.S., Holtgrave, D.R. and Hutton, B.J. (2020) COVID-19 testing, epidemic features, hospital outcomes, and household prevalence, New York state-March 2020. *Clin. Infect. Dis.*, 2020: ciaa549.
26. Menachemi, N., Yiannoutsos, C.T., Dixon, B.E., Duszynski, T.J., Fadel, W.F., Wools-Kaloustian, K.K., Needleman, N.U., Box, K., Caine, V., Norwood, C. and Weaver, L. (2020) Population point prevalence of SARS-CoV-2 infection based on a statewide random sample-Indiana, April 25-29, 2020. *Morb. Mortal. Wkly. Rep.*, 69(29): 960-964.
27. Jamaluddin, M., Hassan, M.K. and Miah, M.M. (2010) Identifying Livelihood Patterns of Ethnic Minorities and Their Coping Strategies Different Vulnerabilities Situation in Chittagong Hill Tracts Region, Bangladesh, Final Report CF No. 7(08).
28. Mollalo, A., Vahedi, B. and Rivera, K.M. (2020) GIS-based spatial modeling of COVID-19 incidence rate in the continental United States. *Sci. Total Environ.*, 728: 138884.
29. Khunti, K., Singh, A.K., Pareek, M. and Hanif, W. (2020) Is ethnicity linked to incidence or outcomes of COVID-19? *Br. Med. J.*, 369: m1548.
30. Yang, X., Yu, Y., Xu, J., Shu, H., Liu, H., Wu, Y., Zhang, L., Yu, Z., Fang, M., Yu, T. and Wang, Y. (2020) Clinical course and outcomes of critically ill patients with SARS-CoV-2 pneumonia in Wuhan, China: A single-centered, retrospective, observational study. *Lancet Respir. Med.*, 8(5): 475-481.
31. Guan, W.J., Ni, Z.Y., Hu, Y., Liang, W.H., Ou, C.Q., He, J.X., Liu, L., Shan, H., Lei, C.L., Hui, D.S. and Du, B. (2020) Clinical characteristics of Coronavirus disease 2019 in China. *N. Engl. J. Med.*, 382(18): 1708-1720.
32. Jehi, L., Ji, X., Milinovich, A., Erzurum, S., Rubin, B., Gordon, S., Young, J. and Kattan, M.W. (2020) Individualizing risk prediction for positive COVID-19 testing: Results from 11, 672 patients. *Chest*, 158(4): 1364-1375.
33. Zhang, J.J., Dong, X., Cao, Y.Y., Yuan, Y.D., Yang, Y.B., Yan, Y.Q., Akdis, C.A. and Gao, Y.D. (2020) Clinical characteristics of 140 patients infected with SARS-CoV-2 in Wuhan, China. *Allergy*, 75(7): 1730-1741.
34. Pan, A., Liu, L., Wang, C., Guo, H., Hao, X., Wang, Q., Huang, J., He, N., Yu, H., Lin, X. and Wei, S. (2020) Association of public health interventions with the epidemiology of the COVID-19 outbreak in Wuhan, China. *J. Am. Med. Assoc.*, 323(19): 1915-1923.
35. Vardavas, C.I. and Nikitara, K. (2020) COVID-19 and smoking: A systematic review of the evidence. *Tob. Induc. Dis.*, 18: 20.
36. Cai, H. (2020) Sex difference and smoking predisposition in patients with COVID-19. *Lancet Respir. Med.*, 8(4): 20.
37. Furman, D., Hejblum, B.P., Simon, N., Jovic, V., Dekker, C.L., Thiébaud, R., Tibshirani, R.J. and Davis, M.M. (2014) Systems analysis of sex differences reveals an immunosuppressive role for testosterone in the response to influenza vaccination. *Proc. Natl. Acad. Sci. U. S. A.*, 111(2): 869-874.
38. Ma, Y., Xu, Q.N., Wang, F.L., Ma, X.M., Wang, X.Y., Zhang, X.G. and Zhang, Z.F. (2020) Characteristics of asymptomatic patients with SARS-CoV-2 infection in Jinan, China. *Microbes. Infect.*, 22(4): 212-217.
39. Kim, G.U., Kim, M.J., Ra, S.H., Lee, J., Bae, S., Jung, J. and Kim, S.H. (2020) Clinical characteristics of asymptomatic and symptomatic patients with mild COVID-19. *Clin. Microbiol. Infect.*, 26(7): 948.e1-948.e3.
40. Hussain, M., Jabeen, N., Raza, F., Shabbir, S., Baig, A.A., Amanullah, A. and Aziz, B. (2020) Structural variations in human ACE2 may influence its binding with SARS-CoV-2 spike protein. *J. Med. Virol.*, 92(9): 1580-1586.
