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Original Article

Detection of Avian Influenza Virus (AIV) in Layer Birds of Selected Districts of Bangladesh using AIV Antigen Rapid Test Kits

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ABSTRACT

Avian influenza (AI) is a highly infectious and emerging viral disease cause substantial economic losses and has the public health concerns. Hence, an attempt was taken to detect the occurrences of Avian influenza virus (AIV) using AIV antigen (ag) rapid test kit in layer birds of commercial poultry farm in selected area of Bangladesh. The study was conducted during January to December, 2020. A total of 97 tracheal swab samples were collected from the clinically suspected avian influenza (AI) infected dead layer birds immediately after postmortem and allowed to AIV ag rapid test kit. The overall AI occurrence by AIV Ag rapid test kit was 59.79%. Particularly, the highest proportion of positive cases was from Tangail district (78.57%), during the 2nd quarter (73.68%) of a year, farms having less than 1500 birds (66.67%) and the layer birds of 22 or less weeks age (63.64%). The mean number of birds (2063.45±1238.28) was higher in positive cases farm than the result showed negative (2035.64±996.39). Similarly, the higher body weight (gm) of Layer bird was for negative cases (1704.36±341.20) to Avian influenza virus without any significant ($p>0.05$) difference with the positive case (1608.10±427.83). Nevertheless, the mortality rate was higher in positive case (9.57±10.52) than the negative case (7.74±7.55) without any significant difference ($p>0.05$). To the sum up, the small-scale layer farms having the layer birds of younger aged are susceptible to AIV especially from April to June of the year.

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Introduction

Avian influenza (AI) is a highly infectious and potentially devastating disease, predominantly of wide range of poultry species even though in human (Alexander, 2000). The Avian influenza (AI) in poultry are caused by influenza A viruses (IAVs) of the Orthomyxoviridae family, which are negative-sense, single-stranded RNA viruses (Capua and Alexander, 2006). The virus envelop contains two surface proteins namely haemagglutinin (HA) and the neuraminidase (NA), which have 16 (H1-H16) and 9 subtypes (NI-N9). Genetically, IAVs has the diverse serotypes due to the blending of these two surface proteins of IAVs (Fouchier et al., 2005). Thereafter, based on lethality for chicken, the IAVs has been classified into two, one is highly pathogenic avian influenza (HPAI) and another is low pathogenic avian influenza (LPAI) (Fouchier et al., 2005). HPAI is a major pathogen causing high mortality in a variety of avian species and is capable of causing sporadic human infections and mortality (Swayne and Suarez, 2000). Though the waterfowl are the natural reservoir of IAVs, AI can easily blowout by the movement of domestic live

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birds, personnel (contaminated shoes and clothing), and contaminated vehicles, equipment, feed, and bird cages (Swayne and Suarez, 2000). In Bangladesh, the first identified HPAI outbreak in poultry was in March 2007 by National Reference Laboratory for Avian Influenza (NRL-AI) after passing a stretched instant risk period which was reconfirmed by the International Reference Laboratory in UK and a regional laboratory in Thailand (Alam et al., 2010). In the interim, several outbreaks have been occurred in different poultry sectors, and caused massive economic loss (Hamid et al., 2016) in the small-scale poultry sector a. In Bangladesh, open wetlands are commonly used by large numbers of migratory waterfowl, native birds and domestic ducks during the whole winter season. These agro-ecological environment act as an important event for making the interaction between the migratory waterfowl and domestic poultry, magnifying the risk of IAVs (Cappelle et al., 2014). Besides this, the small-scale commercial poultry farms having the poor bio-security are extensive throughout the country in addition to household rural chicken. Many of them keep the chickens and domestic semi-scavenging ducks on same premises which may acts as a potential risk factors for transmission of IAVs to commercial poultry farm (Ahmed et al., 2012). However, most of the investigation on Avian influenza virus (AIV) infection in Bangladesh has focused on wild birds, live bird markets, backyard or commercial farms of different poultry species (Bari et al., 2009; Hassan et al., 2015 and Khan et al., 2018) relying to report suspected outbreaks of HPAI. Limited study was in the investigation of AI in Layer birds. Particularly, the commercial layer farming with high yielding strains of chickens has expended rapidly throughout the country. Moreover, an update information on diseases is required for implementing the control and prevention strategies (Meher et al., 2021). Although the various laboratory techniques are existing for the detection AIV, it is thought that use of AIV Ag rapid test kits to detect the AIV is one of the most effective techniques (Rahman et al., 2012). Hence, an attempt was taken to determine the occurrence of avian influenza virus using AIV ag rapid test kit in commercial layer farms in Bangladesh.

Materials and Methods

Study Locations

The Five districts with high poultry density namely the Tangail, Mymensingh, Gazipur, Netrakona and Narsingdi of Bangladesh was considered as study location. These districts lie between the latitudes of 24.30°N to 24.88°N and longitudes of 90°16′4″E to 90.73°E. The software ArcGIS-ArcMap version 10.8 (ESRI, USA) was used to prepare the study map (Figure 1) showing the study sites. The study was conducted during the period from January to December, 2020.

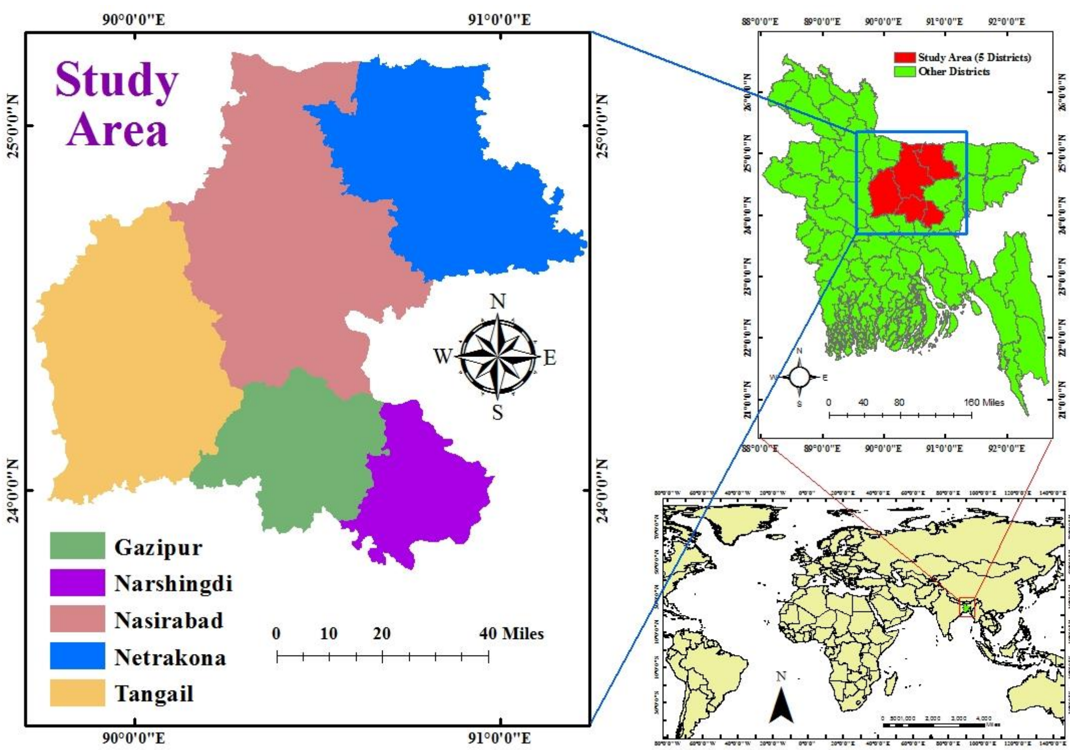

Figure 1. Spatial location of the avian influenza virus occurrence study area of Bangladesh

Collection of samples

In this study, a total of 97 tracheal swab samples were collected from the dead layer birds immediately after postmortem of the dead birds from the poultry farms situated in the study location. The poultry that exhibit all the postmortem lesions caused by the avian influenza virus, only allowed for the sample collection. After collection of samples, placed aseptically in separate sterile plastic vial containing 1 mL viral transport media (VTM) and maintained in ice carrier and then tested in the lab immediately. No ethical permission was required to conduct this study.

Clinically and gross pathologically detection of Avian influenza in layer birds

At first, the specific body parts, such as comb, wattle, legs, shanks, beaks were inspected prudently and changes were recorded. After a systemic dissection of the organs of dead birds, the changes in the organs were noted and the changes were compared with the findings of (Bari et al., 2009) to suspect clinically the avian influenza cases. The sterilized separate set of instruments were used for each case to perform the postmortem examination and the methods applied which followed by the previous methods (Hossain et al., 2018).

Data Collection

The epidemiological information of the respective farm was collected in a structured questionnaire was deployed during sample collection. Farmer's consent was taken before the data collection. The farm size was divided into three categories according to the number of birds namely birds of <1500, 1500 to 3000 and >3000 in number. Similarly, the age category of bird was divided into five namely ≤ 20, 21 to 40, 41 to 60, 61 to 80 and >80 of weeks.

Avian Influenza Virus Rapid Ag kit Test

The samples were tested by the avian influenza virus Ag test kit (AIV Ag test card, Shenzhen Lvshiyuan Biotechnology Co. Ltd, China). All the samples were tested according to manufactures instructions. In brief, the top portion of the sticks (tracheal swab) were inserted into the buffer bottle and was securely mix the swab until the sample has been dissolved into the diluent. Then the cap of bottle was screwed and agitated for 10 seconds by shaking vigorously to ensure good sample extraction. The whole reagents were returned to room temperature before running the assay. A disposable dropper was used to aspirate 4 to 5 drops of sample mixture and were dispensed into the sample well of rapid test kit. The results were obtained within 3 to 5 minutes but observed for 15 minutes. The result was observed by naked eye. The appearance of only single band at "C" mark indicates the negative result (Figure 2). On the other hand, the presence of two bands at "T" and "C" mark indicates a positive result (Figure 2).

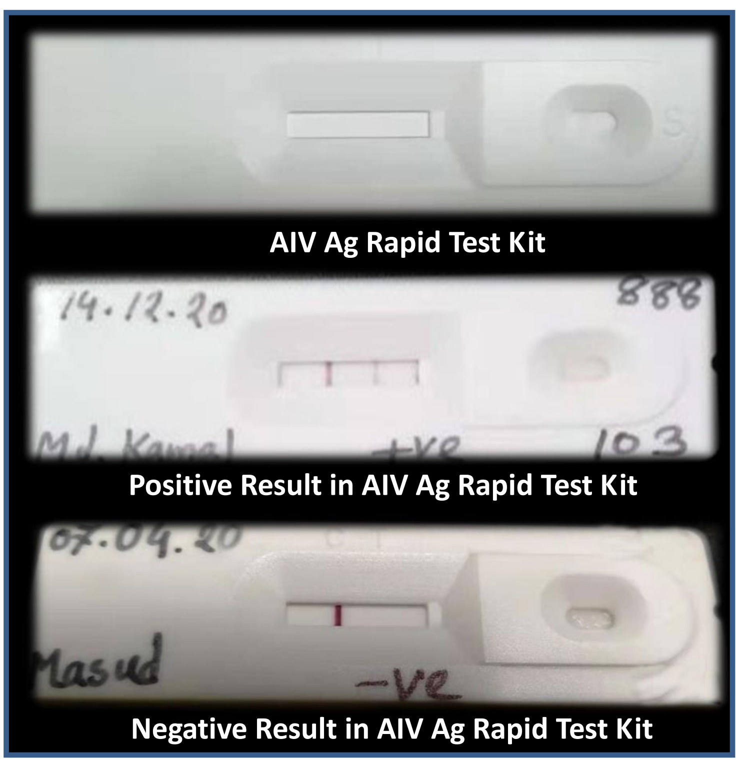

Figure 2. Avian influenza virus Ag test kit (AIV Ag test card) with positive and negative result

Statistical Analysis

All the data and test result were organised into a spread sheet (Microsoft Excel-2010) and then transferred into Statistical Package for Social Sciences (SPSS) version 25.0 for further analysis. The Pearson's Chi-square test was performed to determine the association between the categorical variables. As applicable, the mean of different variable was analysed by using individual t-test and one-way ANOVA. Before performing the all-statistical test, all the assumption for specific test was checked and found them fitted. The p-values less than 0.05 were considered statistically significant.

Results

The distribution of avian influenza in different district of selected area is presented in Table 1. A total of 97 samples from clinically suspected cases were collected from the tracheal swab of dead layer birds. Among the five districts, the significantly (p<0.01) highest proportion of sample was 37.11% from Mymensingh district. Nevertheless, the lowest proportion was 11.34% from the Netrakona district. Thereafter, the Table 1. Shows the result of AIV Ag rapid test of the clinically suspected samples, where the positive and negative cases was 59.79% and 40.21% respectively. Surprisingly, the highest positive case was 78.57% in Tangail district and lowest 50.00% in Mymensingh district. But the result of AIV Ag rapid test was not significantly differ among the study district.

Table 1. Occurrence of Avian influenza in Layer birds of different district

District	Observed cases		*p* value	Negative Case		Positive Case		*p* value
	N	%		N	%	N	%	
Mymensingh	36	37.11	0.001	18	50.00	18	50.00	0.322
Gazipur	17	17.53		8	47.06	9	52.94	
Tangail	14	14.43		3	21.43	11	78.57	
Netrakona	11	11.34		3	27.27	8	72.73	
Narsingdi	19	19.59		7	36.84	12	63.16	
Total	97	100.0		39	40.21	58	59.79	

As is presented in Table 2, the samples of different quarter of a year (2020). In this way, the significantly (p<0.05) highest sample was 36.08% in the 1st quarter and the lowest was 17.53 in the 3rd quarter of the year. However, the highest and lowest positive case was 73.68% and 54.29% in 2nd and 1st quarter of the year respectively without any significant (p>0.05) association.

Table 2. Occurrence of Avian influenza in Layer birds according to four quarter of a year

Quarter of Year	Observed cases		*p* value	Negative Case		Positive Case		*p* value
	N	%		N	%	N	%	
1st	35	36.08	0.042	16	45.71	19	54.29	0.568
2nd	19	19.59		5	26.32	14	73.68	
3rd	17	17.53		7	41.18	10	58.82	
4th	26	26.80		11	42.31	15	57.69	
Total	97	100.00		39	40.21	58	59.79	

The Table 3, shows the occurrence of avian influenza in Layer birds according to farm size and age categories of layer birds. According to the farm size, the percentage of clinically observed cases was significantly highest (43.30%) in the farm where the birds were 1500 to 3000 in number having the mean of 2187.86±389.49. Interestingly, the lowest proportion (16.49%) was in the farms having more than 3000 birds and the mean was 4079.38±949.86, significantly (p<0.001) higher than others farm sizes. On the other hand, 66.67% was the highest for positive cases in AIV Ag rapid test, which was in the farms of less than 1500 birds having the mean of 1074.62±204.99. In the side of age group, though the significantly (p<0.001) highest clinical cases were observed in the layer birds of 21 to 40 weeks age (42.27%), but the highest proportion of positive case in AIV Ag rapid test was 63.64% in the layer birds of 22 weeks and or less. The mean of the number of layer birds in different age group had the significant (p<0.001) differences where the highest mean was 2074.55±1060.47 and this was in the group of layer birds having the age of ≤ 20 weeks. In this age group the minimum and maximum range was 300 - 4000 number of layer birds.

Table 3. Occurrence of Avian influenza in Layer birds according to farm size and age category

Variable	Level	Observed case N	Observed case %	p value	Negative Case N	Negative Case %	Positive Case N	Positive Case %	p value	Mean ± SD	p value	Range
Farm size (Number of birds)	<1500	39	40.21	0.002	13	33.33	26	66.67	0.412	$1074.62^{c}\pm204.99$	<0.000	300–1470
	1500 to 3000	42	43.30		20	47.62	22	52.38		$2187.86^{b}\pm389.49$		1500–3000
	>3000	16	16.49		6	37.50	10	62.50		$4079.38^{a}\pm949.86$		3200–7000
Age (Weeks)	≤ 20	11	11.34	<0.000	4	36.36	7	63.64	0.995	$2074.55^{e}\pm1060.47$	<0.000	300–4000
	21 to 40	41	42.27		16	39.02	25	60.98		$2142.8^{d}\pm1263.81$		500–7000
	41 to 60	33	34.02		14	42.42	19	57.58		$1955.91^{c}\pm1105.52$		1000–5000
	61 to 80	8	8.25		3	37.50	5	62.50		$1976.25^{b}\pm1031.16$		870–3840
	>80	4	4.12		2	50.00	2	50.00		$2010^{a}\pm958.68$		1200–3400

As is observed in Table 4, the mean ± SD of different parameters for avian influenza positive and negative cases of Layer birds in AIV Ag rapid test. Among them, the insignificantly higher mean number of birds (2063.45±1238.28) was in avian influenza positive cases of farm than the negative case (2035.64±996.39). The mean age for positive case and negative case was 40.12±19.26 and 42.72±23.40 weeks respectively. Though the mean age was higher in positive case but had no any significant difference with the negative case.

Table 4. Avian influenza positive and negative cases of Layer birds in relation to mean ± SD different parameters

Parameters	Mean ± SD			*p* value	Range
	Negative case	Positive case	Total		
Number of birds	2035.64±996.39	2063.45±1238.28	2052.27±1141.75	0.907	300–7000
Age (Weeks)	42.72±23.40	40.12±19.26	41.16±20.94	0.552	2–135
Average body weight (gm)	1704.36±341.20	1608.10±427.83	1646.80±396.25	0.243	150–2450
Mortality/day	7.74±7.55	9.57±10.52	8.84±9.44	0.353	1–60

The monthly distribution of avian influenza clinically observed and AIV Ag rapid test positive cases are presented in figure 3. As is observed the avian influenza clinically observed cases and positive cases in AIV Ag rapid test showed almost the similar trend in different months of 2020. Though the clinically observed cases touched the pick at March about 18 cases, but positive cases in AIV Ag rapid test was culminated in the month of December amounting more than 8 cases. Nonetheless, clinically observed cases felt down pointedly in the month of June and August, which was lowest cases (4). Unlike the clinically observed cases, the positive cases in AIV Ag rapid test remained steady with little fluctuation from July to October counting the 2 to 4 positive cases. Though the positive case in rapid test had no remarkable fluctuation, but sharply dropped down in June, which was the lowest (less than 2). However, the two lines (clinically observed cases and positive cases in AIV Ag rapid test) had slow upward trend in the month of December.

However, the clinically affected Layers were suddenly fallen in ill with mild gasping, reduced in feed and water intake. Thereafter, the four major clinical condition was observed, those were cyanotic combs and wattles, oedema of the head and face, lethargy and huddling and ecchymotic mark of the leg shanks. On the other hand, after opening up the carcasses, the congested and hemorrhagic musculature appeared along with there was petechial hemorrhages at the mucosa of respiratory tract (RT) specially the upper RT. Moreover, the hemorrhagic exudates were present in larynx and trachea, congestion in trachea, lungs, liver, spleen, heart, and kidney also observed. More often the air sacs appeared to cloudy and opaque. Egg peritonitis with ruptured of egg follicles was the predominant lesion observed in laying birds.

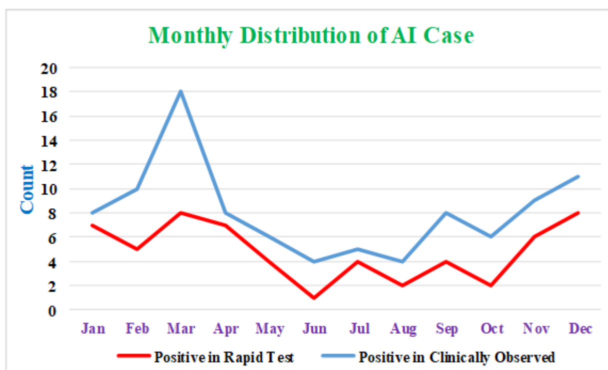

Figure 3. Monthly distribution of Avian influenza virus prevalence in selected study area

Discussion

In this study, the occurrence of avian influenza was determined from the clinically observed cases by allowing the sample in AIV Ag rapid test. In AIV Ag rapid test, the occurrence was 59.79%, which is similar to a previous study conducted in Bangladesh where the authors, Hassan et al. (2020) and Ansari et al. (2016) reported that the avian influenza seroprevalence was 63.8% and 55.6% respectively. But they used the serological (c-ELISA) and molecular methods of RT-PCR for detection of AIV. Though, all the samples were from clinically suspected cases, but only 59.79% was positive in AIV Ag rapid test. These variations might have some complicated post-mortem lesions of mixed infection and also may there be some factors associated with sample transportation. Basically, the AIV Ag rapid test gives the more accurate result than the clinically suspected result. The clinically observed sample size was highest in Mymensingh. Mymensingh is the largest district among the study area and a lot of small-scale poultry farming was raised in this area (Hossain et al., 2010). The highest positive cases in AIV Ag rapid test were in Tangail district. The Tangail district, one of the most crowded districts for both poultry and human populations at the northern perimeter of Dhaka, the capital of Bangladesh (Parvin et al., 2019). this crowded condition may favor the transmission of avian influenza. Additionally, authors also reported that the morbidity of up to 90% dominated by respiratory disease and reduced egg production in layer in their sampled area where Tangail was the vital (Parvin et al., 2019). Determining the seasonality of avian influenza viruses is important, because circulating avian influenza viruses may leads to reassortment and emergence of novel influenza viruses in human which competent of causing pandemics situation (Gerloff et al., 2014). Though this study observed more cases in January to March but highest positive in rapid test was in summer (April to June). Reports from other studies in Bangladesh and Asia observed a distinct seasonal pattern of highly pathogenic avian influenza incidence that peaked during autumn and winter of the year (Gerloff et al., 2014 and Khan et al., 2018). The poultry farms having less than 1500 birds were more positive to avian influenza rapid test. The small-scale farmers are less aware to maintain biosecurity practices (Meher et al., 2020) and they have to face more economic hardship during farming. Although, the authors (Bertran et al., 2016) reported that the age is not a determinant factor in susceptibility of avian influenza virus rather than the management factors and biosecurity. Whereas, another author, Hassan *et al.* (2015) reported the highest (25.81 %) occurrence was in duck of 5 months age. However, our study showed that the age had influence on avian influenza. Nonetheless, the other viral diseases which mainly infect the respiratory system, like infectious bronchitis also occurred in higher aged layer birds in Bangladesh (Meher et al., 2017). The variable, body weight of layer bird, was considerably lower in positive case which corroborate the findings of other author (Gharaibeh, 2008) who stated that the avian influenza infected birds had lower body weight resulting from reduction in weight gain due to H9 infection. Moreover, viral infection decreases the feed consumption. Particularly, the viral infection on pancreatic tissue, which results in decrease production of pancreatic enzymes essential for efficient food digestion (D. Silvano et al., 1997). Ultimately, the body weight of infected birds will be decreased. Thereafter, the mortality rate was high in layer bird due to avian influenza virus infection. This finding has the association with the other research where the author Biswas *et al.* (2011) reported that the incidence rates of mortality in commercial layer was 0.0179 per chicken-day at risk. To suspect the clinically observed case for avian influenza virus, the clinical and post-mortem findings that were recorded in this study was in line with the findings of other authors (Bari et al., 2009 and Khan et al., 2018) who stated the clinical and post-mortem findings of avian influenza virus after confirmed by another molecular test.

Limitations

However, this study has some limitations. First, the sample size was lower according to the study area and number of poultry farm. Secondly, only one laboratory techniques were used to confirm the avian influenza virus. Thirdly, the farm management information was not included in this study. Lastly, we used only the one category (Layer) of poultry in this research.

Conclusion

The current study underlines the occurrence of avian influenza virus by AIV Ag rapid test from clinically suspected cases. Nearabout sixty percentages of clinically suspected cases showed the positive result to AIV Ag rapid test. Particularly, the clinical cases from the layer farm of less than 1500 birds, having the age of 22 weeks and or less with lower body weight in Tangail district at 2nd quarter of the year were more susceptible to avian influenza detected by AIV Ag rapid test.

Contribution of the authors

MMM and MA were developed the concept and designed the experiments. NMMH collected the sample and RS performed the laboratory test. MRI and NSIN contributed to record the data. MMM evaluated the result, analyzed data statistically and contributed to writing the manuscript. MA and MMM contributed to revising manuscript critically for important intellectual content. All authors read the article and approved the final version to be published.

Conflicts of Interest

The authors declared that they have no conflicts of interest.

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