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Characterization of hemagglutination activity of emerging Newcastle disease virus in Bangladesh

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Abstract

Aim: Newcastle disease (ND) is an important viral disease for poultry caused by avian paramyxovirus which can be identified by its nature of agglutination activity with red blood cell (RBC) of different species. The study was aimed to characterize the hemagglutinating (HA) activity of ND virus (NDV) at three different temperatures using RBC of five avian species, six mammalian species, and eight different human blood groups.

Materials and Methods: The study was conducted from January to December 2014 at Chittagong Veterinary and Animal Sciences University. Five avian and six different mammalian species were selected for the study. In each species, two blood samples were collected aseptically. Eight different blood groups (A+, A−, B+, B−, AB+, AB−, O+, and O−) were studied in human. HA test was performed using two virus strains ND lasota and field isolate of very virulent NDV (VVNDV) with mentioned species of RBC at chilling (4°C), incubating (37°C), and room temperature (24°C).

Results: Avian RBC requires less time for agglutination than mammalian RBC. Incubation temperature (37°C) requires lowest time and chilling temperature requires highest time for agglutination of RBC. Duck RBC requires lowest time (17.81 min) while chicken RBC needs highest (57.5 min) time for HA at incubation temperature and at chilling temperature, respectively, against ND lasota virus and with field strain. Goat RBC requires significantly higher time for HA (184.68 min) at chilling temperature than other mammalian species. Human RBC requires almost similar time but O+ and O− blood group do not show any HA activity. Significant variation ($p<0.05$) found in quail RBC at incubation temperature. In mammalian species, a significant difference ($p<0.05$) has been observed in goat and horse RBC at chilling; horse and dog RBC at incubation; goat, horse, buffalo, and dog RBC at room temperature. In human, significant variation ($p<0.05$) has been found in A+, A− and B− blood group in chilling, in B+ blood group at incubation and A+, B+, B−, AB− blood group at room temperature against two virus strains.

Conclusion: ND is considered as an economically significant disease which is highly contagious in nature infecting many avian species. The threat of ND outbreak to poultry industry necessitates effective control measures to reduce the burden in commercial and backyard farming in Bangladesh.

Keywords: chilling temperature, hemagglutination, incubation temperature, Newcastle disease virus, Newcastle disease virus lasota strain, very virulent Newcastle disease virus strain

Introduction

For the past five decades, production in poultry industry has documented greater changes than in any other world's livestock subsector in agricultural production. Promising trends in livestock production indicate that the global production of poultry meat and dairy will double by 2050 [1]. Poultry industry is one of the fastest emerging livestock subsectors which has the annual growing rate of around 20% in Bangladesh [2]. The industry plays a key role in the national economy by making employment. Moreover, the sector is committed to mitigate the demand of good quality protein using meat and egg in inexpensive price [3].

Newcastle disease (ND) is a highly contagious viral disease that attacks many species of domestic and wild birds, caused by ND virus (NDV) which belongs to the family *Paramyxoviridae* and genus, Rubulavirus [1,4-6]. Mortality may reach up to 100% in infected chicken [1]. The disease is important considering the number of animals affected each year and the huge economic impact on the poultry production industry [7]. No treatment for NDV exists,

but the use of prophylactic vaccines and biosecurity measures reduces the likelihood of NDV outbreaks. Vaccination has been reported as the only safeguard against endemic ND [8].

NDV is an enveloped virus having two membrane proteins, the hemagglutinin–neuraminidase (HN) protein associated with cell attachment and release, and the fusion (F) protein mediating fusion of the viral envelope with cellular membranes [6,9]. For infectivity and pathogenicity of NDV, both HN and F glycoproteins are important for virus [10] for the production of protective antigens and virus neutralizing antibody responses [11,12]. The F protein has been shown to induce protective immunity to NDV in chickens. However, the birds showed lower neutralizing antibody titers, but HN protein has also been shown to protect birds from virulent NDV challenge and it has also been shown that monoclonal antibodies to F protein neutralize NDV better than monoclonal antibodies to HN protein [12].

Avian paramyxovirus serotype-1 is a member of the *Paramyxoviridae* family and is the causative agent of virulent ND which is capable of infecting a wide range of avian species leading to a broad range of clinical symptoms [13]. It might have capacity to agglutinate the red blood cell (RBC) of mammalian and other birds other than chicken if they have HA protein binding receptors on the membrane of their RBCs. The HN protein is also responsible for different NDV strains to agglutinate type of RBCs [14]. However, all NDV strains agglutinate chicken RBC [15], but additionally, the lentogenic strains agglutinate mammalian RBCs [14,15]. Therefore, the study was aimed to characterize the hemagglutinating (HA) activity of NDV to multi-species RBC at different temperatures.

Materials and Methods

Ethical approval

The study protocol was permitted by the Ethics Committee of the Chittagong Veterinary and Animal Sciences University (CVASU), Chittagong, Bangladesh.

Duration, species selection, and sample collection

The study conducted in CVASU from January to December 2014. Five avian (chicken, duck, geese, quail, and pigeon) and six different mammalian species (cattle, buffalo, sheep, goat, horse, dog, and human) were selected for the study. From each species, two blood samples were collected using antiseptic swab, sterile syringe, and vial containing ethylenediaminetetraacetic acid as anticoagulant. Each sample was replicated for at least 2 microtiter plate means 16 times per sample. In case when human samples were collected, informed consent was attained from each of two participants. Eight different blood groups (A+, A−, B+, B−, AB+, AB−, O+, and O−) were studied in human. After collection blood samples were transported to the laboratory for the preparation of 1% RBC.

Selection of virus strain

Two strains of NDV were selected for the study where one is lasota strain from commercial vaccine (Intervet, The Netherlands). Another is field isolate of very virulent NDV (VVNDV), collected from the Department of Microbiology and Veterinary Public Health laboratory, CVASU. The field isolate previously isolated and stored at −86°C. The stock virus treated with gentamicin after thawing of virus to remove the bacterial contamination. We treated gentamicin 500 μg/mL of inoculum at room temperature for ½ h to inactivate bacteria if present. The sterility of inoculum was checked by adding few drops of inoculum into nutrient agar and blood agar. No growth was observed after 24 h of incubation keeping at 37°C.

Propagating of virus

Both the virus (vaccine and field isolate) were propagated in 9-day-old embryonated chicken eggs which were collected from the hatchery. The eggs were candled and marked inoculation sites. Eggs were placed in an egg rack with the inoculation site uppermost. By using cotton wool and 70% alcohol to swab the end of the eggs to be inoculated and allowed to evaporate the alcohol. Viral inoculum was treated with gentamicin and kept it 20 min before inoculation. Pierced a hole in the end of the egg at the marked inoculation site by a dental drill. By attaching the needle drawn inoculum into 1 ml syringe. 0.1 ml of inoculum was injected into the egg through the hole in the eggshell by keeping the needle and syringe vertical position. The needle was withdrawn from the egg and the hole in shell was sealed with nail polish. After that the used needles and syringes were discarded. The inoculated eggs were placed into incubator for 48 h. The embryo was checked after 24 h of inoculation for any death of embryo. After 48 h of incubation eggs were transferred to chilling temperature for 24 h [16].

Collection of allantoic fluid

After 24 h of chilling, embryonated eggs were placed into a biological safety cabinet. The egg shell was broken on the top by using sterile forceps, opened at the air sac and pulled back the allantoic membrane. The all egg fragments were placed, including parts of the shell into a materials bag. Allantoic fluid was harvested from each egg by using a sterile syringe being careful not to sucked up yolk or blood with the allantoic fluid. All allantoic fluid has been collected (pooled) and aliquot the collected fluid into Eppendorf tubes and freezer at −86°C to use as stock.

Preparation of 1% RBC

Collected blood was transferred to a falcon tube suitable for centrifugation and added phosphate buffered saline (PBS) to fill the tube. The sample was centrifuged at 1500 rpm for 10 min and discarded the supernatant by using a Pasteur pipette. Care was taken not to disturb the pellet of RBCs and centrifugation step was repeated for 3 times. The cells now formed a pellet after being washed 3 times and centrifuged. The

next step was measured and prepared the 1% solution of cells for storage by adding 1 ml packed cell with 99 ml PBS [16].

Preparation of 4 HA unit virus

One HA unit in the HA titration is the minimum amount of virus that will cause complete agglutination of the RBCs. HA test was performed using 1% chicken RBC with two-fold dilution of the virus to detect the concentration of virus. The last well in the 96-well plates that shows the complete HA with 1% chicken RBC contains one HA unit. The well that contains 4 HA unit was identified and calculated the dilution factor. 4 HA unit virus suspension was prepared by adding 1 ml virus with required amount of diluent [16].

HA test

50 μL of PBS was dispensed into each well of the microwell plate up to column 7. 50 μL of test samples (virus) were placed in the first well of each row of column 1. By using a multichannel pipette, two-fold serial dilutions carried out across the plate until column 6. Then, 50 μL of 1% RBCs of tested species was added in each well including column 7. The wells in this column 7 are control wells that contain only PBS and RBCs. Gently tapped the sides of the plate to mix and placed a cover on the plate and allow the plate to stand in three different temperatures (e.g., chilling, room temperature, and incubation temperature). Result was taken at every 5 min after the settlement of RBC in control row. Result was recorded in a data sheet [16].

Data analysis

Obtained data were entered into spreadsheets of the MS Excel-2007 Program. Data were sorted and cleaned in the Excel program before exporting to STATA-11. t-test was performed to compare HA at different temperatures for different species of RBC by two strains of NDV. One-way ANOVA was carried out to compare the time required for HA within species by two virus strains at three different temperatures. A $p<0.05$ was considered statistically significant.

Result

HA activity of avian RBC with ND lasota strain

For ND lasota strain HA was observed in six microwell plates for a single species of RBC at three different temperatures (chilling temperature, incubation temperature, and room temperature) depicted in Table-1. It was observed that in all cases the highest time was required for the HA activity at chilling temperature. The lowest time for HA activity was required at incubation temperature. Among the chilling temperature, the highest time (57.5 min) was recorded in case of chicken and the lowest time (29.06 min) was recorded in case of pigeon. At different incubation temperature, the highest time (24.69 min) was recorded in case of chicken and the lowest time (17.81 min) was recorded in case of duck. At different room temperature, the highest time (32.19 min) was recorded in case of chicken and the lowest time (21.25 min) was recorded in case of duck.

HA activity of avian RBC with VVNDV field isolate

In case of VVNDV among the avian species at chilling temperature, the highest time (56.26 min) for HA activity was recorded in case of chicken and the lowest time (28.59 min) was recorded in case of quail. Among the incubation temperature, the highest time (23.19 min) was recorded in case of chicken and the lowest time (17.81 min) was recorded in case of duck. At room temperature, the highest time (31.25 min) was recorded in case of chicken and the lowest time (22.50 min) was recorded in case of duck shown in Table-2.

HA activity of mammalian RBC with ND lasota strain

In case of ND lasota virus among the mammalian species at all three different temperatures, the highest time for HA activity was recorded in case of goat and the lowest time was recorded in case of horse described in Table-3.

HA of mammalian RBC with VVNDV field isolate virus

In case of VVNDV among the mammalian species at chilling temperature, the highest time (175.94 min) for HA activity was recorded in case of goat and the lowest time (64.06 min) was recorded in case of horse. Among the incubation temperature, the highest time (125.63 min) was recorded in case of goat and the lowest time (52.66 min) was recorded in case of horse. Among the room temperature, the highest time (168.13 min) was recorded in case of goat and the lowest time (58.13 min) was recorded in case of dog shown in Table-4.

HA activity of human RBC with ND lasota virus

In case of ND lasota virus among the human blood group at chilling temperature, the highest time (112.50 min) for HA activity was recorded in case of AB+ and the lowest time (95.93 min) was recorded in case of A+. Among the incubation temperature, the highest time (71.88 min) was recorded in case of B− and the lowest time (55.93 min) was recorded in case of A+ and A−. Among the room temperature, the highest time (85.39 min) was recorded in case of B− and the lowest time (64.69 min) was recorded in case of A+ blood group reflected in Table-5.

HA activity of human RBC with VVNDV

In case of VVNDV among the avian human blood group at chilling temperature, the highest time (109.69 min) for HA activity was recorded in case of AB+ and the lowest time (86.57 min) was recorded in case of A−. Among the incubation temperature, the highest time (69.00 min) was recorded in case of B− and the lowest time (55.63 min) was recorded in case of A−. Among the room temperature, the highest time (75.31 min) was recorded in case of AB+ and the lowest time (60.31 min) was recorded in case of B+ blood group described in Table-6. Among the eight individual blood groups of human RBCs require almost similar time at

Table-1: Time required for HA of avian RBC with NDV (lasota strain) at different temperatures (mean±SD).

Species	Chilling temperature/4°C (min)	Incubation temperature/37°C (min)	Room temperature/24°C (min)	p value
Chicken	57.5±6.06	24.69±4.04	32.19±3.15	0.001
Duck	29.69±3.40	17.81±3.63	21.25±6.0	
Geese	34.06±3.75	21.56±3.01	29.69±4.23	
Pigeon	29.06±3.75	20.31±3.40	24.38±3.60	
Quail	29.68±3.86	22.81±2.56	25.63±3.10	

HA=Hemaglutination, RBC=Red blood cell, NDV=Newcastle disease virus, SD: Standard deviation

Table-2: Time required for HA of avian RBC with VVNDV (field isolate) at different temperatures (mean±SD).

Species	Chilling temperature/4°C (min)	Incubation temperature/37°C (min)	Room temperature/24°C (min)	p value
Chicken	56.25±5.96	23.13±3.10	31.25±3.42	0.001
Duck	29.69±3.35	17.81±3.64	22.50±4.09	
Geese	33.13±3.54	20.94±3.23	28.44±3.52	
Pigeon	29.84±3.91	19.38±3.59	22.81±4.07	
Quail	28.59±3.64	20.63±3.59	24.06±3.28	

HA=Hemaglutination, RBC=Red blood cell, VVNDV=Very virulent Newcastle disease virus, SD: Standard deviation

Table-3: Time required for HA of mammalian RBC with NDV (lasota strain) within different temperatures (mean±SD).

Species	Chilling temperature/4°C (min)	Incubation temperature/37°C (min)	Room temperature/24°C (min)	p value
Goat	184.68±6.18	125.31±6.70	173.75±6.95	0.001
Sheep	78.13±4.43	58.75±3.87	70.00±4.83	
Horse	67.50±4.83	51.25±4.28	56.25±5.32	
Cattle	72.50±4.47	54.38±4.43	62.81±4.64	
Buffalo	71.57±4.37	55.00±4.47	64.06±3.75	
Dog	69.38±4.78	54.38±3.59	64.06±2.72	

HA=Hemaglutination, RBC=Red blood cell, NDV=Newcastle disease virus, SD: Standard deviation

Table-4: Time required for HA of mammalian RBC with VVNDV (field strain) within different temperatures (mean±SD).

Species	Chilling temperature/4°C (min)	Incubation temperature/37°C (min)	Room temperature/24°C (min)	p value
Goat	175.94±6.12	125.63±6.08	168.13±7.27	0.001
Sheep	76.25±4.29	59.22±3.83	67.81±4.47	
Horse	64.06±4.56	52.66±4.40	59.38±4.03	
Cattle	73.75±3.42	53.44±4.99	64.38±3.10	
Buffalo	70.94±5.84	54.69±4.57	60.94±4.91	
Dog	70.00±4.48	52.67±5.39	58.13±5.74	

HA=Hemaglutination, RBC=Red blood cell, VVNDV=Very virulent Newcastle disease virus, SD: Standard deviation

Table-5: Time required for HA of human RBC with NDV (lasota strain) within different temperatures (mean±SD).

Blood group	Chilling temperature/4°C (min)	Incubation temperature/37°C (min)	Room temperature/24°C (min)	p value
A+	95.93±5.83	55.93±4.91	64.69±4.64	0.001
A−	97.18±6.31	55.93±4.91	65.00±4.08	
B+	99.38±9.28	61.25±5.00	69.38±9.70	
B−	96.87±5.12	71.88±4.78	85.39±5.38	
AB+	112.50±7.30	63.75±5.91	75.00±7.30	
AB−	101.56±5.39	62.35±4.71	70.28±5.14	

HA=Hemaglutination, RBC=Red blood cell, NDV=Newcastle disease virus, SD: Standard deviation

a specific temperature but O+ and O− do not show any HA activity against any one of the virus strains.

Comparison of HA activity of avian RBC with two strains of NDV

At chilling temperature, the highest time (57.5 min) for HA activity was recorded in case of chicken and the lowest time (27.55 min) was recorded in case of quail. There was no significant variation of time requirement for HA activity within different avian species. In case of quail, p=0.09 indicating a very close to significant variation of time requirement by VVNDV and ND lasota vaccine strain. At incubation temperature, the highest time (24.69 min) for

HA activity was recorded in case of chicken and the lowest time (17.81 min) was recorded in case of duck. In case of quail, p=0.05 indicates a significant variation of time requirement by VVNDV and ND lasota vaccine strain. At room temperature, the highest time (32.19 min) for HA activity was recorded in case of chicken and the lowest time (21.25 min) was recorded in case of duck. There was no significant variation of time requirement for HA activity within different avian species at room temperature depicted in Table-7.

Comparison of HA activity of mammalian RBC with two strains of NDV

At chilling temperature, the highest time (184.69 min) for HA activity was recorded in case of goat and the lowest time (64.06 min) was recorded in case of horse. In case of goat, the p=0.04 (p<0.05) and in case horse p=0.04 (p<0.05) indicating a very close to significant variation of time requirement by VVNDV and ND lasota vaccine strain. At incubation temperature, the highest time (125.98 min) for HA activity was recorded in case of goat and the lowest time (50.94 min) was recorded in case of dog. In case of horse the p=0.06 indicating a very close significant variation of time requirement by VVNDV and ND lasota vaccine strain. At room temperature the highest time (173.75 min) for HA activity was recorded in Table-8. In case of goat and the lowest time (56.25 min) was recorded in case of horse. In case of goat the p=0.033 (p<0.05), in case buffalo p=0.05 and in dog p=0.0012 (p<0.05) indicating a very close to significant variation of time requirement by VVNDV and ND lasota vaccine strain at room temperature.

Comparison of HA activity of human RBC with two strains of NDV

At chilling temperature, the highest time (112.5 min) for HA activity was recorded in case of AB+ and the lowest time (86.56 min) was recorded in case of A−. In case of A+, A− and B− blood group p<0.05 indicating a very close to significant variation of time requirement by VVNDV and ND lasota vaccine strain. At incubation temperature, the highest time (65.31 minutes) for HA activity was recorded in case of AB+ and the lowest time (54.06 minutes) was recorded in case of A+. In case of B+ and B− and B− blood group p<0.05 indicating a very close to significant variation of time requirement by VVNDV and ND lasota vaccine strain shown in Table-9. At room temperature, the highest time (86.88 min) for HA activity was recorded in case of B+ and the lowest time (60.31 min) was recorded in case of A+. In case of

Table-6: Time required for HA of human RBC with VVNDV (field isolate) within different temperatures (mean±SD).

Blood group	Chilling temperature/4°C (min)	Incubation temperature/37°C (min)	Room temperature/24°C (min)	p value
A+	90.62±4.42	59.06±3.75	69.31±4.27	0.001
A−	86.57±5.98	55.63±5.12	62.81±7.00	
B+	97.50±8.16	58.13±5.74	60.31±5.31	
B−	89.68±4.98	69.00±4.55	74.69±3.40	
AB+	109.69±7.18	65.31±3.90	75.31±5.62	
AB−	105.93±6.11	63.13±4.43	65.63±3.10	

HA=Hemaglutination, RBC=Red blood cell, VVNDV=Very virulent Newcastle disease virus, SD: Standard deviation

Table-7: Comparing time requirement (min) of HA activity of avian RBC with two strains of NDV at different temperatures.

Species	Chilling temperature (4°C)			Incubation temperature (37°C)			Room temperature (24°C)		
	ND lasota	Field isolate	p value	ND lasota	Field isolate	p value	ND lasota	Field isolate	p value
Chicken	57.5±1.51	55.0±1.44	0.24	24.69±1.16	23.13±0.77	0.27	32.19±0.79	31.2±0.83	0.43
Duck	29.68±0.85	29.68±0.85	1.00	17.81±0.90	17.82±0.63	1.00	21.25±1.15	22.5±0.80	0.44
Geese	34.06±0.49	32.19±0.78	0.14	21.56±0.72	20.93±0.81	0.58	29.69±1.07	28.44±0.87	0.37
Pigeon	29.06±0.94	30.63±1.01	0.26	20.31±0.85	19.84±0.61	0.45	24.38±0.90	22.81±1.02	0.26
Quail	29.69±0.96	27.50±0.79	0.09	22.81±0.64	20.62±0.89	0.05	25.63±0.77	24.06±0.81	0.17

HA=Hemaglutination, RBC=Red blood cell, NDV=Newcastle disease virus, SD: Standard deviation

Table-8: Comparing HA of mammalian RBC with two strains of NDV at different temperatures.

Species	Chilling temperature/4°C			Incubation temperature/37°C			Room temperature/24°C		
	ND lasota	Field isolate	p value	ND lasota	Field isolate	p value	ND lasota	Field isolate	p value
Goat	184.69±1.54	175.93±1.52	0.004	125.31±1.67	125.98±1.39	0.778	173.75±1.4	168.13±1.82	0.033
Sheep	78.13±1.11	76.25±1.07	0.233	58.75±0.97	59.69±0.96	0.59	70±1.21	67.81±1.37	0.239
Horse	67.5±1.21	64.06±1.11	0.047	51.25±1.07	54.06±1.04	0.06	56.25±1.33	59.38±1.00	0.07
Cattle	72.5±1.12	73.75±0.85	0.381	54.38±1.11	52.5±1.37	0.29	62.81±1.12	64.38±0.77	0.259
Buffalo	71.56±1.09	70.94±1.06	0.734	55.01±1.12	54.38±1.2	0.70	64.06±0.94	60.94±1.22	0.05
Dog	69.38±1.20	70.00±1.12	0.705	54.38±0.90	50.94±1.6	0.07	64.06±0.67	58.13±1.43	0.0012

HA=Hemaglutination, RBC=Red blood cell, NDV=Newcastle disease virus, ND=Newcastle disease

Table-9: Comparing HA of human RBC with two strains of NDV at different temperatures.

Blood group	Chilling temperature (4°C)/min			Incubation temperature (37°C)/min			Room temperature (24°C)/min		
	ND lasota	Field isolate	p value	ND lasota	Field isolate	p value	ND lasota	Field isolate	p value
A+	95.93±1.45	90.63±1.10	0.007	55.93±1.22	54.06±0.93	0.23	64.69±1.16	60.31±1.06	0.00
A−	97.19±1.57	86.56±1.49	0.00	55.93±1.22	55.63±1.28	0.86	65.0±1.02	62.81±1.77	0.29
B+	99.38±2.32	97.5±1.53	0.54	61.87±1.0	58.12±1.43	0.04	69.68±1.24	60.31±1.32	0.00
B−	96.88±1.28	89.69±1.24	0.00	72.5±0.91	69.06±1.13	0.02	86.88±1.28	74.68±0.85	0.00
AB+	112.5±1.83	109.69±1.8	0.28	63.13±1.43	65.31±0.96	0.22	74.06±1.52	75.32±1.40	0.55
AB−	105.93±1.52	105.93±1.52	1.00	62.5±1.2	63.12±1.10	0.70	70.12±1.29	65.63±0.77	0.00

HA=Hemaglutination, RBC=Red blood cell, NDV=Newcastle disease virus, ND=Newcastle disease

A+, B+, B− and AB− blood group $p<0.05$ indicating a very close to significant variation of time requirement by VVNDV and ND lasota vaccine strain.

Discussion

ND is still scrutinized as an economically significant disease which is highly contagious nature in infection for many avian species throughout the globe [17,18]. The study was conducted to characterize the HA activity of NDV by using different species of avian and mammalian RBC at three different temperatures. The HA activity of NDV is concerned with virus identification and measurement of antibody [19]. Their thermostability, the agglutination of mammalian erythrocytes, the rate of elution from chicken erythrocytes, the responsiveness to monoclonal antibodies, and the inhibition by lectin-binding were all investigated to classify NDV strains [17,20].

Among the species, it has been observed that avian RBC require less time for agglutination than mammalian RBC. It may due to the presence of nucleus in avian RBC which makes it easy settle down within short time than mammalian RBC. On the other hand, the test can be used to characterize NDV in rapid and cheapest way [14].

The research reveals that incubation temperature (37°C) require lowest time to generate HA activity irrespective of species, chilling temperature need highest time for agglutination of RBC and room temperature require time in between incubation and chilling temperature. Similar observation was also recorded by Hussain *et al*. [21] and Awad *et al.* [22] in avian influenza virus and by Hawkes [23] in hepatitis B. The mechanism underlying this observation of indifferences in agglutination activity of virus strain possibly associated with the variable level of enzymatic activity that is optimum at incubation temperature (37°C) and decreases with decreasing temperature [24].

Both ND lasota and virulent field isolate NDV agglutinate all avian and mammalian RBC with variable time requirement. It has been found that among avian RBC, highest agglutination time was recorded in chicken (57.5 min) at chilling temperature and lowest time was recorded in duck (17.81 min) at incubation temperature. In case of mammalian RBC highest time was recorded in goat (184.68 min) at chilling temperature and lowest time was recorded in horse (51.25 min). Similar observation was described by Hussain *et al*. [21], Awad *et al*. [22], Couceiro *et al*. [25], Ibu *et al*. [26], Adebayo [27], but there was strain variability in agglutination. Adair *et al*. [28] and Lu *et al*. [29] found that some avian and mammalian viruses agglutinate the erythrocytes of certain animals. In the study, Ibu *et al*. [26] found that five virus strains (Bn2, Bn8, Herts, Kudu, NCD) failed to agglutinate bovine and equine RBC. This HA property reflects the fact that such erythrocytes possess receptors for certain surface components of the virus particle that function as cell attachment protein. The cell receptors for paramyxoviruses (NDV) are HN is a multifunctional molecule. HN is responsible for the attachment of virus to receptors containing sialic acid expressed by different complex gangliosidic structures as GD 1a, GT 1b, and GQ 1b [30,31] which could be abundant on cell surfaces of erythrocytes originated from specific animal origins.

In case human RBC among the eight different blood groups (A+, A−, B+, B−, AB+, and AB−) show complete agglutination of RBC by ND lasota and VVNDV except O blood group which does not show any agglutination by both virus strains. Couceiro *et al*. [25] found similar result but he found variation in HA activity in case of human "O" blood group that may be attributed to lack of any surface antigen in human "O" blood group which showed the occurrence of variability in the amount and specificity of erythrocyte receptors in paramyxovirus [25,32]. Comparing the time requirements to show HA activity among different species of RBC with two NDV strains at different temperatures have also been studied. At chilling temperature, there was no significant variation of time requirement for HA activity in between two strains of NDV. At incubation temperature, there was significant difference ($p=0.05$) observed in quail this may be due to strain variability of NDV that do not recognize the sialic acid receptor exhibited by same RBC [25]. At room temperature, there was no significant variation found in two strains of NDV.

The variability in the pattern of agglutination of certain mammalian erythrocytes by the field virus and lasota strains demonstrated herewith. The pattern of variability as observed could be of theoretical and

practical significance. Among mammalian species at chilling temperature in case of goat the p=0.04 ($p<0.05$) and in case horse p=0.047 ($p<0.05$) indicating significant variation of time requirement by VVNDV and ND lasota vaccine strains. At room temperature, significant variation was observed in goat (p=0.033), buffalo (p=0.05), and dog (p=0.0012) that might be due to some intra-strain differences are still apparent in terms of their hemagglutinability patterns [26]. The selective ability of mammalian RBCs as a tool for differentiation between natural and vaccinal strain in a serological test has been reported by Adebayo [27].

In human RBC, at chilling temperature in blood group A+ [p=0.007), A− (p=0.00), and B− (p=0.00), $p<0.05$ indicating a significant variation of time requirement by VVNDV and ND lasota vaccine strains. At incubation temperature in blood group B+ (p=0.04) and B− (p=0.02) and at room temperature in blood group (A+, B+, B− and AB−), $p<0.05$ indicating a significant variation of time requirement by VVNDV and ND lasota vaccine strains. The variation in agglutinability of most mammalian erythrocytes by the velogenic strains appears to agree with the findings of Haruna *et al*. [33]. These workers observed a direct linkage between the agglutinability of mammalian RBCs and virulence of NDVs. A similar observation evidenced earlier [34]. The latter authors concluded that the agglutination of erythrocytes of different animal species could serve as a quick means of identifying and separating different viruses as well as different strains of one and the same virus.

The fact that mammals are not natural hosts of NDV in addition to their availability and easy management make them good candidates or substitutes for specific pathogen-free chickens in the supply of indicator systems for Hemagglutination Inhibition titrations. However, Adebayo [27] observed that some erythrocyte indicator systems differed in their sensitivity to the detection of ND antibody.

Conclusion

ND remains a constant threat to the poultry industry, limiting disease for poultry producers worldwide. The variety of clinical presentations and the emergence and spread of new genetic variants make recognition and diagnosis challenging. Presently, we confirm that the NDV of vaccine and field strain were agglutinate the avian, mammalian and human RBC at three different temperatures with variable time except human "O" blood group which is not agglutinated by any one of the virus strains. In every species of RBC, incubation temperature requires less time for HA than room temperature while chilling temperature requires higher time for HA than other two temperatures. Avian species required less time for HA than mammalian species. A significant variation of time requirements for HA activity also observed in some species and human blood group. It is recommended that various factors such as source of erythrocytes, type of diluent, incubation time, and pH variation can be evaluated with their potential effects on HA activity.

Authors' Contributions

HU: Conception and design, sample collection, laboratory works, data analysis and interpretation, review of the manuscript. KI: Assist in laboratory testing, analysis and interpretation of the data, writing and drafting the manuscript, critical review of the manuscript. MB: Assist in drafting, designing, cross checking and critical review of the manuscript, reference list crosschecking. SI: Reference list crosschecking and review of the manuscript. AA: Overall supervision, conception and design, instruction and review of analysis and critical review of the manuscript. All authors read and approved the final manuscript.

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Competing Interests

The authors declare that they have no competing interests.

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