Research Article

Comparative Efficacy of Newcastle Disease's Live Vaccines in Broilers Using Hemagglutination Inhibition (HI) Test at Jaba Mansehra

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Abstract | Two commercial vaccines against the Lasota strain of Newcastle disease (ND), the Lasota vaccine-1 and vaccine-2, and two vaccines against the Mukteswar strain of Newcastle disease, the Mukteswar vaccine-1 and vaccine-2, were assessed for their effectiveness and impact on the productivity of broilers. 75 days old broiler chicks overall were divided into five equal groups and given the following labels: A, B, C, D, and E. Each group was then split up into pens with a maximum of five birds in each pen. On days 14 and 21, the birds in groups B, C, D, and E received active immunisation against ND using Lasota-1, Lasota-2, Mukteswar-1, and Mukteswar-2, respectively, leaving A as the control group left uninfected. On days 21 and 35 of the study, the serum HI antibody response to these four immunisations was assessed. As evidence of its strong efficacy, Group C, which had received the Lasota (vaccine-2) vaccination, displayed high antibody titers throughout the experiment. In terms of geometric mean titers, there was a significant difference (p 0.05) between the groups. On days 7, 14, 21, and 35 of the experiment, data on other performance indicators, including as feed intake, water intake, total body weight, and feed conversion ratio (FCR), were gathered. Throughout the course of the trial, there were significant differences in these performance metrics (p 0.05). There was no discernible difference in the productive performance of the broilers between the vaccinated groups, and the unvaccinated broilers (control group) performed better in terms of weight growth and FCR than the vaccinated groups. According to the data, group C of broilers given Lasota vaccine-2 proved to be more effective in terms of producing antibodies in broilers, while unvaccinated broilers performed better in terms of productivity compared to the vaccinated flock. However, it was advised that the decision on which vaccine to use depend not only on the aforementioned elements but also on the specifics of a given region, including the organization of veterinary services, prior knowledge, population distribution, communication infrastructure, and climate.

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Introduction

It is difficult to find food these days given how quickly the population is expanding. One issue worth mentioning is the rising global need for food. For many years, the poultry industry has been a reliable source of high-quality food all over the world. Even today, to close the gap between global food demand and supply, the poultry industry has transitioned from modest rural flocks to more sophisticated commercial broiler farming, even in developing nations like Pakistan (Sajid *et al.*, 2015). But there are certain factors that have come up against the poultry industry including infectious viral as well as bacterial diseases which are hampering its progress. Newcastle disease, caused by Newcastle Disease Virus (NDV), member of Avian Paramyxo virus type 1 (APMV-1) of genus Avula virus and family Paramyxoviridae. This is one such challenge to the poultry industry in the developing countries like Pakistan (Islam *et al.*, 2019). On the basis of pathogenicity, strains of NDV are generally grouped as highly (velogenic), moderately (mesogenic) and weakly pathogenic (lentogenic). There have been reports that just 5% of field strains in Pakistan are velogenic, whereas the remaining 95% are either mesogenic or lentogenic (Waheed *et al.*, 2013). Newcastle disease has led to huge economic losses associate with high morbidity, high mortality and many other production related losses. It is epidemologically evident that NDV is being evolved continuously and its isolates have been classified so far into more than 18 distinct genotypes (Bello *et al.*, 2018). NDV may be fatal sometimes and birds don't show any symptoms till death or the symptoms may range from mild air succulitis to severe nervous and visceral damages, leading to paralysis and even death of the bird. In Pakistan, annual incidence is reported to be up to 38% among the broilers and mortality has been up to 50% that may varies according to the pathogenicity of the strain (Sajid *et al.*, 2015). Different methods have been implemented to avoid, reduce and prevent the disease on national level in birds flocks (Makoui and Feizi, 2014). Vaccination is currently one of the most prevalent methods to overcome Newcastle disease (García-Sastre and Mena, 2013). The Lasota strain, which is a lentogenic vaccine, is used for the initial immunisation in Pakistan's vaccination protocol. This is followed by the application of the Mukteswar and Komorav strains (Sajid *et al.*, 2015). In most cases, the Mukteswar vaccine is manufactured by incorporating the necessary amounts of mineral oil, emulsifier, and required antigen into particles that are covered in surfactant (Mahboob *et al.*, 1999). When chicks reach the age of eight weeks, it is recommended that they begin receiving Mukteswar vaccines in order to augment the immunity established by lentogenic or Lasota vaccines. According to the reports, the Mukteswar vaccine, which is manufactured in Pakistan, is not highly pathogenic nor highly immunogenic (Khurshid and Rehman., 2018). It has been reported that primary vaccination at day 7 followed by secondary dose at day 28 is best regarding immune response and protection potential (Islam *et al.*, 2019).

The level of maternally produced antibodies, the age of the chicken at the time of vaccination, and the type of vaccine all have an effect on the immunological response of the broiler chicken to the antigen of the vaccine (Abbas *et al.*, 2006). Day old chickens are unable to respond well to vaccination due to high level of maternally derived antibodies. So, a secondary vaccination dose is required to achieve the level of immunity as the level of antibodies fall gradually with age (Rehan *et al.*, 2019). It is suggested to vaccinate the young chicks before 12 days of age; otherwise, the decline in maternal antibodies can make bird more susceptible to infection (Sajid *et al.*, 2015). The HI test is the immunoassay that is utilized most frequently for the detection of NDV antibodies in poultry. The purpose of this test is to identify any antibodies that inhibit the ability of chicken red blood cells to bind to the Hemaglutanin Neuraminidase (HN) protein that is produced by NDV. It is generally accepted that infection exists when there is a discernible rise in the level of HI antibodies in the blood (Choi *et al.*, 2015). Despite being so useful in reducing and combating Newcastle disease, according to a report, vaccination leads to reduced body growth, less body weight resulting in lower Feed Conversion Ratio (FCR) compared to non-vaccinated birds (Sajid *et al.*, 2015). The growth promoting effect of propolis extract and vaccinated with NDV vaccines showed the highest increase in body weight followed by those received NDV vaccines and control negative groups respectively (Hegazi *et al.*, 2012).

Despite being so widely studied about, Newcastle Disease is still prevalent in Pakistan, posing huge threats to poultry farming. The purpose of present

experiment was to compare the efficacy and potency levels of ND vaccines; Lasota (international) and Mukteswar (national) of different companies in broiler chickens using the Hemagglutination (HI) test and also their impact on productive performance of the broilers.

Materials and Methods

Housing management

The Poultry Research Institute Jaba in Mansehra was the location where the research was conducted in order to evaluate the comparative efficacy of Newcastle Disease's live vaccines in broilers using the Hemagglutination Inhibition (HI) test as well as their influence on growth performance, which included feed intake (FI), water intake (WI), weight gain (WG), and feed conversion ratio (FCR) of the broilers. In the beginning, the house was tidied up, whitewashed, and thoroughly fumigated for the purpose of disinfection before the newborn chicks were brought inside. Rice husk was used as a litter with a depth of around 2 to 4 inches to provide the broilers with suitable bedding. Throughout the course of the experiment, the temperature of the room was kept at approximately 68 degrees Fahrenheit, and the humidity was kept at approximately 55% to 60%. The appropriate biosecurity precautions were taken into consideration.

Experimental birds

For the purpose of carrying out this research, a neighbourhood hatchery was contacted and asked to provide the participants with 75 days old broiler chicks of the Cobb-500 strain. On the day the chicks arrived at the Poultry Research Institute Jaba, they were given a weight and then randomly divided into five groups: Group A, Group B, Group C, Group D, and Group E. Each of these groups contained 15 birds. Each of the five groups was separated into one of the five pens. After that, each batch was cloned a further three times, with each replica carrying a total of five baby chicks (Table 1).

Vaccines

The dietary and managemental settings, including feeding, temperature, ventilation, and humidity, were exactly the same for all of the groups. During the course of the experiment, a free-flowing supply of food was provided to each of the groups. The immunisation against Newcastle Disease Virus (NDV), which was the strain, and the manufacturing business that was responsible for the vaccines were the factors that differentiated the groups. After vaccination with NDV, all of the experimental groups received the virus itself.

Group A

Chicks belonging to Group A were not given any vaccines at any point during the experiment because they were considered to be part of the control group.

Group B

On day 7 and day 21 of the experiment, Lasota strain Vaccine-1 of NDV was administered through the drinking water.

Group C

On days 7 and 21 of the trial, the Lasota strain Vaccine-2 of NDV was given to the chicks in group C through their drinking water.

Group D

On days 7 and 21 of the trial, the Mukteswar strain Vaccine-1 of NDV was given to the chicks in group D through their drinking water as a preventative measure against NDV.

Group E

On days 7 and 21 of the trial, the Mukteswar strain Vaccine-2 of NDV was given to the chicks in group E through their drinking water as a preventative measure against NDV. (Vaccine-1 and Vaccine-2 is used for the different manufacturing companies of the NDV Vaccine).

Table 1: *Study design of the experiment with subgroups (replicates).*

	Group A (Control)	Group B Lasota 1	Group C Lasota 2	Group D Mukteswar 1	Group E Mukteswar 2
Subgroup 1	5	5	5	5	5
Subgroup 2	5	5	5	5	5
Subgroup 3	5	5	5	5	5

Data collection

After the birds had been brought in, they were given a weight, and then they were randomly split up into the five different groups. After that, the birds were weighted on a regular basis on days 7, 14, 21, 28, and 35 using a weight balance, and the average weight of the birds in each group was recorded. On days seven, twenty-one, twenty-eight, and thirty-five, the amount of food consumed by birds of all groups was tallied. The amount of feed that was delivered was computed, and at the conclusion of the day, the amount of feed that was still in the feeder was also weighed. The amount of food eaten can be calculated as the difference between the amount of food that was supplied and the amount of food that was still in the feeder the following day. The amount of food eaten by each bird was determined by dividing the total amount of food consumed by a group by the number of chicks in each group (Aji *et al*., 2011). On days 7, 21, 28, and 35, observations were made on the feed conversion ratio. In order to calculate the FCR, we used the following formula: FCR = Feed supplied/ Animal weight gain (Aji *et al*., 2011). A random selection was used to choose three chicks for each group, and one chick from each replica was used to collect blood from the chicks by inserting sterile needles into the vein in the chick's wing on days 14, 21, 28, and 35 of the experiment, respectively. After being collected, the blood samples were deposited into clot activator glass tubes manufactured in Germany by BD, and then they were sent to the laboratory of the Poultry Research Institute (PRI) in Jaba to have their serums separated. After centrifuging the blood samples at 2500 rpm for 15 minutes at room temperature, the supernatant fluid was extracted with a sterile pipette and placed in the serum collection plastic tubes that were supplied by the PRI Jaba. The HI tests for NDV were carried out with 10 HA units of antigen in V-bottom microplates utilising automated equipment (CanalcoAutotiter III, Canalco, Inc., 5645 Fisher Lane, Rockville, Maryland 20852). To make the serial two-fold dilutions of serum in antigen, the starting concentration was 1:5, and the volume used per well was 50l. After adding 50 l of chicken erythrocytes at a concentration of 0.5% to each well of the plate, it was left to incubate at 25 degrees Celsius for one hour before the results were read. The samples that showed a certain central button-shaped settling of RBCs were recorded as positive, and the greatest dilution of each sample that caused haemagglutination inhibition was regarded to be the end point in the experiment. In each individual serum sample, the HI titer was calculated as the reciprocal of the serum dilution. On a weekly basis, one hundred percent of the birds from each experimental group had their geometric mean HI titers determined (Allan and Gough, 1974: Numan *et al*., 2005).

Statistical analysis

The information obtained in this manner was subjected to analysis by means of the analysis of variance (ANOVA) procedure within the context of a completely randomized design (CRD) with two components (Steel *et al*., 1997). The Duncan's Multiple Range (DMR) test was utilized in order to conduct a comparison of the means (Duncan, 1955).

Results and Discussion

Feed intake

The amount of feed that each of the five groups consumed was tracked every week. It was determined, based on the acquired results, that there was not a discernible difference in the amount of food consumed by any of the groups. As can be seen in Table 2, the findings of the experiment revealed that there was a statistically significant difference (p 0.05) between the groups for the entirety of the study.

Table 2: *Feed intake (g/bird) of broilers throughout the experiment.*

Week	Group A	Group B	Group C	Group D	Group E	P value
1st	175±3	175±2	174±4	175±3	171±4	<0.05
2nd	369±5	363±6	364±5	365±7	364±5	<0.05
3rd	607±9	598±8	598±8	599±9	598±8	<0.05
4th	814±13	806±14	808±15	807±13	806±15	<0.05
5th	1092±22	2090±23	1088±21	1089±22	1088±21	<0.05

Water intake

The amount of water used by each of the groups was tracked on a weekly basis. According to the findings, there was not a discernible difference between any of the groups in terms of the amount of water that was consumed by the broilers that were selected. As can be seen in Table 3, there was a statistically significant difference (p 0.05) between the groups with regard to the amount of water that they consumed over the course of the study. This was found among the groups.

Table 3: *Water intake (ml/bird) of broilers throughout the study period.*

Week	Group A	Group B	Group C	Group D	Group E	P value
1st	366±3	359±5	358±5	356±5	361±3	<0.05
2nd	760±6	751±7	748±6	749±7	757±5	<0.05
3rd	1134±12	1125±13	1124±12	1126±14	1131±13	<0.05
4th	1574±15	1564±16	1563±14	1562±16	1571±15	<0.05
5th	2206±21	2202±22	2201±23	2202±21	2203±22	<0.05

Total body weight (g/bird)

The results of weekly measurements of total body weights of all the groups showed significant difference ($p<0.05$) throughout the study period. According to the recorded results, the control group of broilers had maximum body weight as compared to the other groups of broilers that were vaccinated throughout the study duration as shown in the Table 4.

Table 4: *Total body weight (g/bird) of broilers throughout the study period.*

Week	Group A	Group B	Group C	Group D	Group E	P value
0	44±2	44±2	44±2	44±2	44±2	<0.05
1st	183±7	180±5	179±6	181±5	178±6	<0.05
2nd	495±9	485±8	483±8	482± 9	481±8	<0.05
3rd	894±12	880±11	879±12	878±13	881± 12	<0.05
4th	1433±15	1413±16	1412±14	1414±15	1411±16	<0.05
5th	2039±21	2007±22	2010±21	2011±23	2008±22	<0.05

Feed conversion ratio

The feed conversion ratio (FCR) of all the groups was recorded on weekly basis. According to the results, control group of the broilers had better FCR as compared to the other groups of broilers that were vaccinated. There was a significant ($p<0.05$) difference recorded in FCR among the groups throughout the study periodas shown in the Table 5.

Geometric mean titer

(p 0.05) difference in GMT across all of the groups over the entirety of the trial, as displayed in Table 6. At the 21st and 35th day of the experiment, the geometric mean titer (GMT) of each of the groups was measured and reported. On day 21 and day 35, the data that were obtained showed that Group C had a better GMT in comparison to the other groups. There was a really important.

The purpose of this research was to evaluate the effectiveness of two different live vaccinations for Newcastle disease in broiler chickens: Lasota (International) and Mukteswar (National). The evaluation was done with the Haemagglutination Inhibition (HI) test. In the current investigation, there were a total of five different groups of broilers that were given the NDV vaccinations Lasota (Vaccine-1 and Vaccine-2) and Mukteswar (Vaccine-1 and Vaccine-2), respectively. These groups were A, B, C, D, and E. The firms known as Vaccine-1 and Vaccine-2 are both involved in the production of vaccines. According to the data that was collected, the unvaccinated group (which served as a control) experienced a greater increase in weight (6068.60) than did the vaccinated groups. The Lasota (Vaccine-2) group had the best results in terms of weight gain (5985.53) among the vaccinated groups. This was in comparison to the Lasota (Vaccine-1) group (5948.52), the Mukteswar (Vaccine-1) group (59712.02), and the Mukteswar (Vaccine-2) group (5977.11). The findings of the current study are consistent with those of Alexander *et al.* (2004), who found that the weight gain of vaccinated birds was significantly lower than that of non-vaccinated birds over the course of the study. In the case of vaccinated birds, the findings of our study are also in line with those of Martinez *et al.* (2018), who came to the conclusion that body weights were considerably greater (p 0.05) in birds that had been vaccinated with Lasota. Rehmani (1996) also published comparable findings, which indicated that the Lasota vaccination is superior to the Mukteswar vaccine in terms of weight increase.

Table 5: *Feed conversion ratio of broilers throughout the study period.*

Week	Group A	Group B	Group C	Group D	Group E	P value
1st	1.25±0.068	1.28±0.032	1.28±0.054	1.28±0.074	1.27±0.044	<0.05
2nd	1.17±0.032	1.18± 0.041	1.19±0.047	1.21±0.045	1.21±0.050	<0.05
3rd	1.51±0.032	1.50±0.039	1.50±0.039	1.51±0.035	1.49±0.061	<0.05
4th	1.50±0.028	1.51±0.045	1.51±0.021	1.50±0.039	1.52±0.053	<0.05
5th	1.79±0.028	1.83±0.029	1.81±0.024	1.82±0.029	1.81±0.012	<0.05

Table 6: *Geometric mean titer of broilers of broilers throughout the study period.*

Day	Group A	Group B	Group C	Group D	Group E	P value
21st	00	9.15	9.88	9.71	9.67	<0.05
35th	00	15.22	15.27	14.89	14.51	<0.05

Throughout the course of the trial, the authors of this study found no statistically significant differences (p > 0.05) between the birds that had been vaccinated and those who had not been vaccinated. This suggests that immunisations did not have any influence at all on the total amount of feed that was consumed.

According to the results of a recent study, birds who were not given vaccinations (the control group) had a better feed conversion ratio (1.79 0.028) than birds that had been given vaccinations. The group that was injected with Lasota vaccine-2 had a greater Feed conversion ratio (1.510.021) than the other vaccinated groups, which were administered Lasota vaccine-1 (1.510.045), Mukteswar vaccine-1 and vaccine-2 (1.500.039 and 1.520.053, respectively). Alexander *et al.* (2004) provided support for the current study by demonstrating a lower feed conversion ratio in the case of vaccinated birds compared to uninoculated birds. This finding demonstrated that vaccination reduces the risk of disease transmission. This is due to the heat and stress placed on the birds' bodies as a result of vaccination, which led to an inability on the part of the birds to effectively convert the feed they consumed into weight. Martinez *et al.* (2018), in a study that was conducted in agreement with ours, discovered a substantially greater FCR (p 0.05) in the birds who had not been vaccinated. It's also possible that this happened because the vaccination was given after the maternal antibodies had already died off completely. During the course of the trial, the birds received the vaccination for the very first time on day 21, which is why they were unable to convert the feed into weight in a manner that was more effective. As a result, the FCR was much greater on the 35th day of the trial.

The results of our research demonstrate that Lasota vaccine-2 is effective based on the geometric mean titer (GMT) obtained from the haemagglutination inhibition (HI) test. The recorded results gave higher geometric mean titer (15.27) in the birds that had been inoculated with Lasota vaccine-2. The birds that had been administered with other vaccines showed relatively less titers (15.22, 14.89 and 14.51 respectively), followed by the control group, which showed zero titers throughout the experiment. This low titer could be the result of heat stress and a lack of water, both of which contributed to the synthesis of steroids, which in turn caused an immunological suppression. These findings are in line with those found in the research conducted by Makoui and Feizi (2014), who found that the Lasota vaccine strain produced a higher immune response and titer of antibodies compared to other vaccination strains. In addition, the efficacy of NDV Lasota was described by Winterfield *et al.* (1957) in terms of high antibody titers in comparison to those of other vaccinations. Martinez *et al.* (2018) also analysed the effectiveness of the Lasota strain at different administration times and compared the results (as primary vaccine or after the decay of maternal antibodies). According to the findings, using the Lasota strain after the maternal antibodies had been depleted resulted in a high antibody titer as well as a powerful immunological response to the antigen in question.

Conclusions and Recommendations

It was concluded that group C of broilers administered with Lasota vaccine-2 proved to be more effective in terms of antibody production in broilers, and that unvaccinated broilers had better productive performance as compared to vaccinated flock. These findings were derived from the data that was collected and recorded. There was not a discernible difference in the productive performance of broilers across the different groups within the flock that had been vaccinated.

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Novelty Statement

This research work designed to be conducted on the basis of deficient data on comparative efficacy of these market available vaccines in last five years. The finding of the research may be used as effective tool for planning the vaccination program.

Author's Contribution

Ayesha Bakhtiar: Conceived the idea, Data Collection.
Dr. Sardar Azhar Mehmood: Assisted in result and discussion.
Abdul Rauf Bhatti: Overall Management of the article.
Naqash Khalid: Assisted in data collection.
Dr. Shabir Ahmed: Technical Input at every step.
Mr. Javeed Iqbal: Assisted in Statistical analysis.
Dr. Azra Nadeem: Assisted in Introduction and conclusion.
Waqas Ahmad: Assisted in field and Lab work.

Conflict of interest

The authors have declared no conflict of interest.

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