DOI: https://doi.org/10.53350/pjmhs22169363

ORIGINAL ARTICLE

Comparative Study of the Efficiency of the Inactivated H9n2 Vaccine against Avian Influenza in Broiler Chickens

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ABSTRACT

Aim: Our work is devoted to a serological study of avian influenza disease in broiler chickens, after five different vaccine protocols.

Methods: a total of 500 broiler chicks (day-old) were equitably divided into 5 lots. in each batch a specific vaccination protocol was followed. The relative level of serum antibodies against H9N2 virus present in the samples is determined at days: 1, 10, 24, 33, and 51 of age.

Results: for batches 1 and 2: the kinetics of the antibodies present a stable curve not associated with significant modifications ($p > 0.05$) during the entire experimental period, whereas for batches 3, 4 and 5: a peak is observed at D 33 for the first two batches and at D51 for the last

Conclusion: the development of active immunity conferred by vaccinations has not been proven because in groups 3, 4 and 5 the titers of antibodies reach high values only in the case of response to the provoked infectious condition and not following immunization by vaccines.

Keywords: antibodies, ELISA method, immunity, serotype H9N2, vaccine.

INTRODUCTION

The H9N2 virus is widely distributed in nature[1] and is regularly isolated from wild birds and occasionally from pigs and other mammalian species. These viruses usually cause mild disease and became panzootic in the mid-1980s in chickens, ducks, turkeys, pheasants, quails, ostriches and migratory birds[2]. However, and despite the low pathogenicity of H9N2 viruses, their prevalence in poultry around the world causes significant economic losses in the poultry industry[3] and even more serious, it offers many opportunities for the acquisition of mutations[4] , with the risks of causing major problems, especially that this subtype have been observed to cause infection in people[5].

In Algeria, the low pathogenic H9N2 avian influenza virus has been endemic in the poultry industry[6]; therefore, vaccination of broiler chicks has been recommended by health authorities. However, the various commercial vaccines available and which are generally administered to chicks at different ages (from 1 day to 10 days of age) and at reduced doses do not show any proof of their effectiveness on our farms[7]. Our work is devoted to a serological study of avian influenza disease (AI) in broiler breeding. Our objectives are as follows: to assess the immune status of the animals and to compare the kinetics of antibodies specific to the low pathogenic avian influenza virus, according to the vaccine protocol applied to five different batches of broilers.

MATERIALS AND METHODS

Study Area and birds: Our experiment was carried out in five different commercial broiler farms located in the Wilaya of Mila (eastern Algeria). In each building, a total of 100 one-day-old (Arbor acre strain) broiler chickens were housed at the broiler facilities and reared in two-tier cages at a maximum density of 20 birds per cage. Each cage was equipped with wood shavings as bedding material. The chicks from the different hatcheries were subjected to serological and bacteriological control. The study lasted 5 months, from August to December 2020.

Experimental design: In the five experimental flocks (1, 2, 3, 4, and 5), a specific vaccination protocol was followed. The samples required for the assays of serum antibody levels against H9N2 come from batch of 20 chicks chosen at random from each farm. The level of antibodies was determined by the ELISA method at days: 1, 10, 24, 33, and 51 of age (age of slaughter in Algeria: > 50 days). The birds had ad libitum access to feed and drinking water. The health status of the flocks was monitored daily. The experimental vaccine protocol is as follows

In (group 1), 1st day: Turkey Herpesvirus (HVT) vectored Newcastle disease (ND) vaccine and live vaccine against IBD (Infectious bursal disease) by subcutaneous injection. In addition to a live attenuated vaccine by nebulization against IBV (Infectious Bronchitis Virus). 15th and 20th day nebulized live attenuated vaccine against ND

In (group 2), the applied vaccination protocol is the same as that of group 2. Except for the HVT vectored ND vaccine which is replaced by inactivated vaccine against avian influenza virus (H9N2) and ND.

In (group 3), no vaccination protocol was applied

In (group 4), the vaccination protocol applied is the same as that of group 2

In (group 5), the vaccination protocol applied is the same as that of group 1. In addition, on the 10th day the animals were vaccinated with the inactivated vaccine against avian influenza virus (H9N2) and ND.

On the 17th day of the experiment, chicks from each of the groups: 3, 4 and 5, were experimentally contaminated with the H9N2 virus. The diagnosis of H9N2 avian influenza disease is based on clinical and lesional signs. The lesions concern the sinuses, the bronchi, the lungs, the air sacs, and the intestines. These lesions include mucopurulent or caseous inflammation and thickening of the air sacs, serous edema, and other localized lesions[8]. The direct ELISA technique was performed using enzyme immunoassay kits developed by IDEXX® for the detection of avian influenza virus antibodies from chicken serum. The purpose of the assay is to measure the relative level of antibodies present in the serum of test chickens against the avian influenza virus antigen. The relative level of serum antibodies present in the sample is determined by calculating the ratio between the levels of the sample and that of the positive control (S/P). A rate greater than 0.50 is considered positive (presences of antibodies against serotype H9N2).

Statistical analysis: Statistical studies are carried out using the software Graph Pad Prism 7.00. All the results of the serum levels of the antibodies H9N2 in birds of the five experimental groups are expressed as (mean ±SD). The effect of fixed factors: vaccine protocol (lots 1, 2, 3 4 and 5) and sampling day (D1, D 10, D 24, D33 and D 51) and their interaction, on the kinetics of antibodies against the H9N2 virus was analyzed using the ANOVA test (Analysis of Variance) with two factors (vaccination protocol and period). The Tukey or Sidak multiple comparison post test (depending on the type of result) was conducted to test the

significance between the means of the different subgroups. Differences were considered significant when p<0.05.

RESULTS

Table1: Serum levels of antibodies against the H9N2 influenza virus in birds of the five experimental lots during the different sampling periods.

	Day1	Day10	Day24	Day 33	Day 51	Lot effect
Lot 1	269,95±187,42	104,25±117,69	690,20±549,62	715,15±346,68	495,50±165,78	ns
Lot 2	553,45±233,23	92,35±132,43	446,10±277,64	922,05±365,04	918,65±481,34	ns
Lot 3	575,70±347,58	120,00±165,81	509,15±559,56	4369,67±3636,18	2412,67±1677,91	p< 0.05
Lot 4	569,77±284,40	84,25±92,29	564,75±428,04	5026,00±2562,50	3975,11±1671,04	p<0,0001
Lot 5	465,25±256,05	114,35±77,46	1419,60±989,29	3981,25±2010,64	5049,85±2567,48	p<0,0001
Day effect	ns	ns	p<0,0001	p<0,0001	p<0,0001	

Day 1: we note that the birds of the five batches express a serum level of non-protective antibodies testifying to the total absence of innate immunity of maternal origin.

Day10: the general averages remain below the protective threshold,

Between D10 and D24: we observe for batches 1, 2, 3 and 4 a slight non-significant increase (p>0.05). Lot 5 shows a very significant increase (p<0.01) during this same period.

For batches 1 and 2: the kinetics of the antibodies shows a stable curve not associated with significant modifications (p>0.05) throughout the entire experimental period.

For batches: 3, 4 and 5: a peak is observed at D 33 for the first two batches and at D51 for the last. Indeed, the values recorded on D33 are the highest (p<0.001) is noted in batch 3 as well as batch 4. It should be noted that the positive response of batches 4 and 5 is associated with the challenge provoked on D 17.

DISCUSSION

The low pathogenic H9N2 avian influenza virus is becoming a serious threat to poultry. Indeed, H9N2 is an emerging respiratory problem, and which would have a zoonotic potential[9] . Despite the high incidence of H9N2 subtype virus in neighboring African countries, little information is available regarding the circulation of this virus in Algerian poultry flocks. However, co-nfections including IBV, avian Metapneumovirus (aMPV) subtype B, avian influenza virus and Mycoplasma gallisepticum (Mg) were confirmed in poultry flocks manifesting respiratory signs with high mortality[10]

In our study, the possible interference between the passive immunity which the chick inherits from its mother[11] and the development of active immunity following the administration of vaccine against serotype H9N2 justifies the choice[12] of batches lacking protective antibodies on day 1. Furthermore, the results obtained clearly show that H9N2 vaccination of day-old chicks is insufficient to trigger a remarkable immune response, this could be due to an incomplete development of the immune system in day-old chicks[13].

In groups 3, 4 and 5, the immune response due to infection by the virus was confirmed by clinical signs in the form of lesions linked to this disease as well as by a mortality rate of around 97% in the 3rd batch, 45% at the 4th and 50% at the 5th. The development of active immunity conferred by vaccinations has not been proven because in these groups the antibody titers reach high values only in the case of a response to the provoked infectious state and not following immunization with vaccines[14]. The high mortality rate observed in the unvaccinated batch has been confirmed by several studies which show that the risk of superinfections increases significantly[15]. Furthermore, it should be noted that in addition to the vaccine protocols adapted[16] to our farms in Algeria, the development of an effective biosecurity system[17] is essential, because this system is considered the first line of protection against the introduction of all poultry diseases and in particular against emerging diseases.

CONCLUSION

According to the results of the experiment, the H9N2 vaccination should be adapted to our breeding conditions, because the serum level of antibodies remains unsatisfactory and would only reach protective titers around the 50th day of the chicks' life, which coincides with the slaughter period. Moreover, vaccination according to the current programs if it decreases the level of excretion of the wild virus, it does not prevent the infection of the vaccinated poultry.

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