SEROLOGICAL STUDY OF *AVIAN ENCEPHALOMYELITIS* IN LAYING HENS AND ECONOMIC IMPACT OF VACCINATION IN ALGERIA

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Abstract: To determine if *AE* virus disease in laying hen farms in Algeria and to know the economic impact of vaccination, During the period from May 2014 to June 2015 ,sixteen farms with history of lowered egg productivity. 480 serums samples were tested for antibodies using commercial (Elisa) kits. For statistical analysis, Mann-Whitney test was for economic analysis, costs-benefits. Serological results revealed a percentage of 81.25% of positive seroconversion. At 5% degree of significance, economic analysis showed that vaccination against *AE* virus engenders economic benefits.

Key words: Avian encephalomyelitis, Hens, Economic, Vaccination, Algeria

INTRODUCTION

Avian encephalomyelitis (AE) virus infection in laying bird causes unapparent infection or drops in egg production. From December 2013 to March 2014, a preliminary study has been conducted to reveal the suspected etiologies causing egg drop. For this reason, a survey has been performed in order to know the opinion of the veterinary practitioners and their knowledge about the probable etiologies and also the common applied vaccination programs. For this, a number of 400 veterinary practitioners have been selected randomly among 7000 ones working in the different farms of the Algeria. The results showed that 88% of the questioned veterinary practitioners say that viral etiologies are the main causes of egg drop. The aim of this study is to demonstrate the presence of the *avian encephalomyelitis* (*AE*) virus disease in laying hen farms of Algeria from one side, and to analyse the economic impact of vaccination against that virus from the other side.

MATERIAL AND METHODS

From May 2014 to June 2015, 16 laying hen farms spread across the country. These farms have a capacity of 4800 up to 130,000 laying hens, observed by sentinel veterinarian after an egg drop; a data sheet for the laying hens was established containing the following information:The capacity strains, vaccination programs applied, accidents laying observed (percentage drop, external appearance of eggs), the associated clinical symptoms, autopsy examinations and mortality rates recorded during an episode of the egg drop.

Blood samples at the wing vein according to the method of Campbell (1995) were performed on 15 hens per farm right at the moment of the egg drop (S1) and another sample made three weeks later (S2), a total of 450 serums were made. Technically, a volume of 3 ml of blood / chicken was taken at the wing vein and collected in dry tubes and directly centrifuged (3000 rpm / min, 6 min).

After centrifugation, a volume of 300ul plasma was collected using a capillary pipette and divided into two Eppendorf tubes labeled for each sample and kept cold in a freezer (-20 ° C) until the serological analysis performed at the analytical laboratory (LAB-

VET in partnership with the laboratory of BIO CHENE-VERT-France). The kits are supplied by Bio Check (Check Bio BV Holland), they contain 05 plates and all reagents required (ready to use). Each sample was diluted to 1/500 1ul adding 0.5 ml dilution buffer. Measuring results of the ELISA test is performed with a microplate reader: with a spectrophotometer at 405 nm filter. Mann-

Whitney test is a nonparametric test and it is based on the comparison between the medians of the two samples. The null hypothesis (H0) is given by: H0: Median 1 = Median 2. The acceptance of the null hypothesis would signify that the distribution of the variable of interest (virus concentration in serums) is the same in the two serological samples, and then the two samples come from the same population. For economic analysis, costs-benefits comparison approach was used in order to evaluate gains and losses due to vaccination against *AE* virus. Currency conversion from Algerian Dinars (DZA) to Euro (€) was done relying on the current exchange rate (1 € = 120 DZA).The sample size of laying hens that were under vaccination is 10,000 hens.

The applied vaccine consists of bottle that contains 1000 doses and that costs about 30 €. The vaccine against the *AE* is made by eye drop and without a booster at 12 weeks of age. Since one person is sufficient to vaccinate 1000 pullets, 10 people under the direction of a veterinarian were given the task to vaccinate the whole sample.

Estimation of benefits was based on the evaluation of losses in the absence of vaccination. For eggs, the gain calculation was based on the difference between the actual laying hens and the theoretical curve multiplied by the unit price of egg which is 0.06 € and that represents the selling price of the market during the study period. The eggs production shortfall represents the amount of eggs that have been laid by individuals during a clinical episode of *AE* multiplied by the consumption egg price estimated at 0.06 €.

According to the veterinary practitioners questioned in the preliminary study, the number of eggs laid during the manifestation of an egg drop episode is estimated around 9500 eggs for each 10,000 hens. Since reducing the number of subjects who would have died by the AE during the clinical phase of the disease can be considered as a benefit, this later was calculated by multiplying the number of deaths by the unit price of hen estimated at € 3.75. For the 16 studied farms, the average mortality rate attributed to AE was estimated around 5% during an egg drop episode by the previously questioned practitioners (about 500 hen deaths).

RESULTS

Table 1

The seroprevalence of AE depending on the region

Region	Number of farms	Positive serology		Negative serology	
		Number	%	Number	%
East	3	2	66,67%	1	33,33%
Center	11	9	81,82%	2	18,18%
West	2	2	100,00%	0	0,00%
Total	16	13	81,25%	3	18,75%

Table 2

The seroprevalence of AE depending on the duration of egg drop

Duration of Egg drop	Number of farms	Positive serology		Negative serology	
		Number	%	Number	%
<1 week	1	0	0,00%	1	100,00%
1-2 weeks	5	5	100,00%	0	0,00%
2-3 weeks	6	5	83,33%	1	16,67%
> 3 weeks	4	3	75,00%	1	25,00%
Total	16	13	81,25%	3	18,75%

Table 3

The seroprevalence of AE depending on the rate of egg drop

% of Egg drop	Number of farms	Positive serology		Negative serology	
		Number	%	Number	%
5-15%	9	9	100,00%	0	0,00%
> 15%	7	4	57,14%	3	42,86%
Total	16	13	81,25%	3	18,75%

Table 4

Serological results corresponding to the studies farms

Farms	Log10Title				P-values
	Vaccination against *AE*	First Phase Sample (Day 1)	Second Phase Sample (Day 21)	Seroconversion	
F1	No	9428	496	Positive	0,0001
F2	No	427	2315	Positive	0,144
F3	Yes	4177	4643	Positive	0,3
F4	No	8041	8236	Positive	0,772
F5	No	3949	6854	Positive	0,068
F6	No	120	3983	Positive	0,001
F7	No	4723	7829	Positive	0,003
F8	No	5250	5970	Positive	0,3
F9	Yes	121	3194	Positive	0,0001
F10	No	508	7542	Positive	0,0001
F11	Yes	2646	3990	Positive	0,147
F12	No	471	471	Negative	0,135
F13	Yes	294	65	Negative	0,008
F14	No	3781	6338	Positive	0,031
F15	No	4837	5553	Positive	0,561
F16	No	139	124	Negative	0,22

Among the 16 farms selected randomly, 11 were from the center of the country, 3 from the east and 2 from the west. Results concerning the seroprevalence of *AE* depending on the region, the duration of egg drop, clinical symptoms and on the rate of egg drop are presented in tables 1, 2, 3 and 4 respectively. These tables allow us to highlight different results concerning the *AE* in Algeria. Concerning regional side, table 1 shows that positive serology is much higher in the west and the center of the country.

Concerning the duration of egg drop, table 2 shows that manifestation of *AE* in breeding laying hens causes egg drop for periods varying from 1 week to even more than 3 weeks. For farms with egg drop duration less than 1 week, all of them are serologically negative. However, for farms with egg drop duration varying between 1 and 2 weeks, all of them are serologically positive. Positive serology is also high for periods varying between 2 and 3 weeks, and even for more than 3 weeks.

Table 5

Costs-Benefits analysis results of vaccination against AE

Costs		Benefits	
More expenses	**Price in €**	**More products**	**Price in €**
Cost of vaccine (10,000 doses)	300	Eggs production gain	570
Cost of Vaccination (10,000 laying hens)	700	Mortality reduction	1875
Less expenses	**Price in €**	**Less expenses**	**Price in €**
None	0	Treatment reduction	1066,66
Total	**1000**	**Total**	**3511.66**
Benefits – Costs		**2511.66**	
Benefits /Costs		**3.51**	

The clinical signs, for farms presenting them, consist of nasal discharge, infra orbital edema, moderate respiratory illness with conjunctivitis, tracheitis and renal impairment. According to table 3, we notice that *AE* positive seroprevalence is very high in both farms with and without clinical signs (80% and 80.33% respectively), which imply a clear no difference between farms concerning the symptomatically side and the onset of *AE* in breeding laying hens. According to table 4, we notice that all farms (100%) with egg drop rates varying between 5 and 15% present positive seroprevalence.

However, only 57.14% of positive seroprevalence is recorded in farms with egg drop rates exceeding 15%. Results concerning serological analysis of the studied farms are presented in table 5. According to this table, 75% of farms are not subject to vaccination against the *AE*. Serological results revealed a percentage of 81.25% of positive seroconversion, namely in farms F1, F2, F3, F4, F5, F6, F7, F8, F9, F10, F11, F14 and F15.

This seroconversion is clearly noticed by the increase of the number of antibodies in those farms in day 21 compared to day 1. The p-values resulting from the Mann-Whitney test corresponding to the 16 farms are presented in table 5. According to this table, we notice that, at 5% degree of significance, seven farms present significant p-values (those that are < 0.05). This means that, in these farms, we reject the null hypothesis of median equality between the two serological samples and we statistically conclude that those samples do not have the same distribution. For economic analysis, results of costs-benefits analysis are presented in table 7. We notice from this table that vaccination against *AE* virus engenders much more economic benefits than costs. In other words, economic benefits of vaccination against AE are about 3.5 times much greater than costs of vaccination.

DISCUSSION

Given that all other viruses are subject to routine vaccination, *AE* is still not common and vaccination against it is not exhaustively applied in Algeria. This is very clear in our study since 75% of farms are not subject to vaccination against this virus, so positive serology implies necessarily the presence of *AE* in our farms. This is confirmed with Mann-Whitney test results. In fact, since we surely know that samples of day 1 and day 21 come from the same population (the same farm for each two samples); the median inequality between the two samples is not due to the difference of distribution between them, but otherwise to the presence of *AE* virus in laying hens, which implies that the positive seroconversion in the previously mentioned farms is just the consequence of *AE* virus and is not a matter of different populations.

Despite the positive seroconversion, atypical results may be noticed at farm level F1 where there was a drop in the number of antibodies at the second sample compared to the first (table 5). This is due to the very late intervention on laying hens for the first sample phase in farm level F1. It followed therefore the image of a curve in the descending phase of the production of antibodies. It is very clear that *AE* strongly affects the production of egg for consumption and leads to serious economic losses due not only to the drop in production but also because of the resulting fragile and discoloured eggs. Vaccination against *AE* has clearly showed significant economic benefits especially in what concerns egg production gain, hen mortality reduction and hen treatment reduction. Indeed, this microeconomic financial approach allows the conclusion that it is strongly recommended to develop a systematic vaccination of chicken in Algeria. Such procedure would avoid losses estimated at 2511.66 € for each 10000 hens in case of clinical manifestation of *AE*. For comparison work, a lot of studies have been conducted to highlight the presence of *AE* as well as vaccination and vaccination effect. Freitas and Back (2015) demonstrated that clinical, histopathological, and serological evidences obtained in their study showed a significant increase of cases of avian encephalomyelitis in broilers from the last quarter of 2012 until the end of 2013. The cause of this increase was not clear, but vaccination failures were suspected. The monitoring of breeders before the beginning of egg production and the application of two vaccinations, if necessary, were suggested. Roy et al. (2009) showed the presence of *AE* through the diagnosis of the disease based on virus isolation in embryonated chicken's eggs, demonstration of hexagonal virus particles in purkinje cells of cerebellum by electron microscopy and confirmation by agar gel immunodiffusion test using *AE* virus specific antiserum. Asasi et al. (2008) showed the presence of *AE* infection in broiler flocks around Shiraz (Iran) after an increase in serum encephalomyelitis virus antibody titer in affected flocks.

CONCLUSIONS

Throughout this study, we conclude that *avian encephalomyelitis (AE) virus* is present in laying hen farms of Algeria and constitutes a leading risk factor for hen death as well as egg production drop. Without systematic vaccination against this virus, economic losses estimated at 2511.66 € may be encountered for each 10000 hens in case of clinical manifestation of the virus.

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